ANTIBIOTIC SENSITIVITY OF *ESCHERICHIA COLI* ISOLATED FROM ANIMALS AND HUMAN SOURCES

By

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PREFACE

This work has been carried out at the Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, under the supervision of Dr. Suliman Mohammed El Hassan.

DEDICATION

To my parents
With thankful
To my Brothers and Sister
With love
To my colleagues and friends
With wishes

ACKNOWLEDGEMENT

First I would like to thank god for giving me strength, confidence and patience to complete this study.

I whish to express my sincere gratitude to my supervisor Dr. Suliman Mohammed El Hassan for patience supervision, guidance, encouragement, advice, and support.

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ABSTRACT

The objectives of this study were the isolation, identification, examination of antibiotic sensitivity and comparing the size of plasmids DNA of *Escherichia coli*.

A total of one hundred and ninety samples were collected from Khartoum State from infected poultry tissues, calf fecal samples, mastitic cow's milk, women urine, bovine abscesses, and child diarrhea.

E.coli was isolated from infected poultry tissues (81 %), calf fecal sample (60 %), mastitic cow's milk (60 %), women urine (53.3 %), bovine abscess (20) %, and child diarrhea (66.7%).

Twelve antibiotics were examined. All isolates were highly sensitive for amikacin (99%), ceftizoxim (99%), cephalexin (95%), nitrofuranation (95%), gentamicin (89%), ampicillin (85%), chloramphenicol (73%), ciprofloxacin (78%), norfloxacin (78%), ofloxacin (78%). The isolates were resistant to tetracycline (78%) and nalidixic acid (58%). Amoxicillin (61%) and pefoxacin (73%) were medium sensitive.

The infected poultry isolates were completely sensitive to amikacin (100%) and ceftizoxim (100%), while child diarrheal isolates were completely sensitive to cephalexin (100%), nitrofuranation (100%), nalidixic acid (100%) and ofloxacin (100%). Also women urine isolates were completely sensitive to amikacin (100%), ceftizoxim (100%), chloramphenicol (100%), nitrofuranation (100%) and pefloxacin (100%).

Mastitic cow's milk isolates were completely sensitive to all antibiotics (100%) except amikacin and tetracycline showed some resistance (67%).

Calf fecal isolates were completely sensitive to all antibiotics (100%) except amoxicillin, tetracycline, ciprofloxacin, nalidixic acid and norfloxacin.

Bovine abscess isolates were completely sensitive to all antibiotics (100%) except amoxicillin, tetracycline, nalidixic acid, ofloxacin showed high resistance (100%), while ciprofloxacin and norfloxacin showed intermediate susceptibility (50%).

Multi- drug resistance was shown by most of the *E.coli* strains isolated in this study. The multiple drug resistance observed varied from two drugs multiple resistance (tetracycline- nalidixic acid) to nine drugs multiple resistance (cephalexin, gentamicin, ampicillin, amoxicillin, chloramphenicol, ciprofloxacin, norfloxacin, ofloxacin, and pefoxacin).

E.coli strains showed the highest multiple drugs resistance were isolated from infected poultry tissues (up to 9 antimicrobial drugs) women urine (up to 7 antimicrobial drugs) and bovine abscess (up to 6 antimicrobial drugs).

Most of the strains showed multi- resistance; 75.7% of isolates were resistant to at least two antibiotics, 59.8% were resistant to at least three antibiotics, 43.1% were resistant to at least four antibiotics, 25.7% were resistant to at least five antibiotics and 18.9% were resistant to at least six antibiotics.

The isolates did not showed clear bands of plasmid for unseen reason.

ملخص الأطروحة

الغرض من هذه الدراسة عزل وتصنيف الاشريكيا القولونية واختبار مدى حساسيتها للمضادات الحيوية ومقارنة أحجام بلاذميداتها.

تم جمع مائة واثنين وثلاثون عينة فى ولاية الخرطوم من انسجة الدواجن المصابة ومن اسهالات العجول و البان الابقار المصابة بالتهاب الضرع ومن عينات بول نساء مصابات بالتهاب فى الجهاز البولى ومن خراجات الابقارواسهالات الاطفال.

تم عزل الاشريشيا القولونية من انسجة الدواجن المصابة (81%), اسهالات العجول (60%), البان الابقار المصابة بالتهاب الضرع (60%), عينات بول نساء مصابات بالتهاب فى الجهاز البولى(53.3%), خراجات الابقار (20%) ومن اسهالات الاطفال(66.7%).

ايضا تم اجراء اختبار حساسية هذه العزلات لاثنى عشر مضادا حيويا.

كل العزلات(100%) كانت ذات حساسية عالية ل

amikacin (99%), ceftizoxim (99%), cephalexin (95%), nitrofuranation (95%), gentamicin (89%), ampicillin (85%), chloramphenicol (73%), ciprofloxacin (78%), norfloxacin (78%), ofloxacin (78%).

كل العزلات كانت ذات حساسية متوسطة ل

amoxicillin (61%) and co- trimoxazole (60%).

كل العزلات كانت مقاومة ل(78%) tetracycline و(58%) nalidixic acid.

عزلات انسجة الدواجن المصابة لها حساسية كاملة ل

amikacin (100%) and ceftizoxim (100%).

اما عزلات اسهالات الاطفال كانت لها حساسية كاملة ل

cephalexin (100%), nitrofuranation (100%), nalidixic acid (100%) and ofloxacin (100%).

ايضا عزلات بول النساء المصابات بالتهاب فى الجهاز البولى كانت لها حساسية كاملة ل

amikacin (100%), ceftizoxim (100%), chloramphenicol (100%), nitrofuranation (100%) and pefloxacin (100%).

اما عزلات البان الابقار المصابة بالتهاب الضرع كانت لها حساسية كاملة لكل المضادات الحيوية (100%) عدا amikacin and tetracycline اظهرت بعض المقاومة .

عينات اسهالات العجول كانت لها حساسية كاملة لكل المضادات الحيوية عدا amoxicillin,tetracycline, ciprofloxacin, nalidixic acid and norfloxacin.

عزلات خراجات الابقار كانت لها حساسية كاملة لكل المضادات الحيوية عدا amoxicillin, tetracycline, nalidixic acid, ofloxacin اظهرت مقاومة عالية 100% .

اما norfloxacin و ciprofloxacin اظهرت حساسية متوسطة 50%.

المقاومة المتعددة للمضادات الحيوية وجدت فى معظم عزلات الاشريشيا القولونية المعزولة فى هذه الدراسة وهى تتراوح بين المقاومة لمضادين حيويين

(tetracycline- nalidixic acid)

وحتى تسعة مضادات حيوية

(cephalexin, gentamicin, ampicillin, amoxicillin, chloramphenicol, ciprofloxacin, norfloxacin, ofloxacin, and pefoxacin).

عزلات الاشريشيا القولونية التى اظهرت اعلى مقاومة متعددة عزلت من انسجة الدواجن المصابة(حتى تسعة مضادات حيوية) عزلات بول النساء المصابات بالتهاب فى الجهاز البولى(حتى سبعة مضادات حيوية) عزلات خراجات الابقار (حتى ستة مضادات حيوية).

اغلب العزلات اظهرت مقاومة متعددة 75.7% من العزلات كانت مقاومة لمضادين حيويين كحد ادنى, 59.8% كانت لها مقاومة لثلاثة مضادات حيوية كحد ادنى, 43.1% كانت لها مقاومة لاربعة مضادات حيوية كحد ادنى, 25.7% كانت لها مقاومة لخمسة مضادات حيوية كحد ادنى و18.9% كانت لها مقاومة لستة مضادات حيوية كحد أدنى.

عزلات الاشريشيا القولونية لم تظهر حلقات بلاذميدات واضحة لأسباب غير معلومة.

INTRODUCTION

Escherichia coli usually abbreviated to *E. coli*, discovered by Theodor Escherich, a German pediatrician and bacteriologist, is one of the main species of bacteria that live in the lower intestines of mammals. *E. coli* is the type species of the genus *Escherichia*, which contains mostly motile gram-negative bacilli within the family *Enterobacteriaceae* and the tribe *Escherichia.* The bacteria are necessary for the proper digestion of food and are part of the intestinal flora. *E. coli* can be the causative agent of several intestinal and extra-intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and Gram-negative pneumonia. The enteric *E. coli* are divided on the basis of virulence properties into enterotoxigenic (ETEC, causative agent of diarrhea in humans, pigs, sheep, goats, cattle, dogs and horses), enteropathogenic (EPEC, causative agent of diarrhea in humans, rabbits, dogs, cats and horses), enteroinvasive (EIEC, found only in humans), verotoxigenic (VTEC, found in pigs, cattle, dogs and cats), enterohaemorragic (EHEC, found in humans, cattle and goats), attaching-effacing (AEEC, collects *E. coli* found among EPEC in humans, EHEC in humans, cattle and goats, and porcine strains that colonize the gut in a manner similar to human EPEC strains) and enteroaggregative *E. coli* (EAggEC, found only in humans). Appropriate treatment depends on the disease and should be guided by laboratory analysis of the antibiotic sensitivities of the infecting strain of *E. coli*. Antibiotics which may be used to treat *E. coli* infection include (but are not limited to) amoxycillin, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin. Antibiotic resistance is a growing problem. Some of this is due to misuse of antibiotics

in humans, but some of it is probably due to the use of antibiotics as growth promoters in animal's food.

In recent years, the management of *E. coli* infections has been increasingly complicated by the emergence of resistance to most first-line antimicrobial agents (James *et al*., 2006). Antibiotic drug- resistance is worldwide problem. Drug resistance is now very wide spread, and strains are commonly encountered that are resistant to more than one drug (Carter, 1985).

After six decades of widespread antibiotic use, bacterial pathogens of human and animal origin are becoming increasingly resistant to many antimicrobial agents. Antimicrobial resistance develops through a limited number of mechanisms: (a) permeability changes in the bacterial cell wall/membrane, which restrict antimicrobial access to target sites; (b) active efflux of the antimicrobial from the cell; (c) mutation in the target site; (d) enzymatic modification or degradation of the antimicrobial; and (e) acquisition of alternative metabolic pathways to those inhibited by the drug (McDermott et al., 2003).

Numerous bacterial antimicrobial resistance phenotypes result from the acquisition of external genes that may provide resistance to an entire class of antimicrobials. These genes are frequently associated with large transferable extrachromosomal DNA elements called plasmids, on which may be other mobile DNA elements such as transposons and integrand. An array of different resistance genes may accumulate on a single mobile element, presenting a situation in which multiple antibiotic resistance can be acquired via a single genetic event. The versatility of bacterial populations in adapting to toxic environments, along with their facilities in exchanging DNA, signifies that antibiotic resistance is an inevitable biological

phenomenon that will likely continue to be a chronic problem. Successful management of current antimicrobials, and the continued development of new ones, is vital to protecting human and animal health against bacterial pathogens (McDermott et al., 2003).

This study is carried out to isolate *E.coli* from different sources, investigate the prevalence of antibiotic resistance to different antimicrobial agents and compare the size of plasmids DNA of *E.coli* isolates having different multi- resistance.

CHAPTER ONE

1 LITERATURE REVIEW

1.1 Historical background

Escherichia coli commonly called *E. coli*, is just one of many bacteria that can cause diarrhea. The first isolation of *E. coli* was mode by a young Austrian Pediatrician, Dr. Theodor Escherich in Munich 1885. He was holding clinical assistantships at children's Polyclinic and Hunters children s Hospital; he carried out researches in the intestinal flora of children as a possible cause of epidemics of diarrhea. The name of the *Bacterium coli* commune was read for the first time, which is now known as *B. coli*. He became the leading bacteriologist in the field of pediatrics and an authority on infant nutrition (Tortura *et al*., 1986).

In animal the ability of *E. coli* to cause diarrhea was first suggested in late 1800 and early 1900 by several veterinary workers studying the calves scours (Nocard and Lectainche, 1898 ; Joest , 1903 : Titze and Orcut, 1908 .

The organism was isolated along with other bacteria from the faeces of newborn babies. It was found to be concomitant with breastfeeding. Escherich described it as a short plump rod, growing readily on gelatin or agar. On potato and coagulated milk, it grew as a slimy mass with the production of acid. In the early days different names have been applied to the organism such as *Bacillus escherichii* in 1889, *Bacillus coli* in 1895. In 1900 it was known variously as *Bacterium verus*, *Bacillus coli communes* and *Aerobacter coli*. The genus Escherichia was first proposed by Migula in 1895 and became firmly established in 1919 by castellani and charmers in the third edition of the Manual of Tropical Medicine (Tortura *et al*., 1986).

Bray (1945) investigated and outbreak of enteritis in London hospital and showed that aparticular serological type of *E. coli* was the epidemic agent. In the same year certain strains of *E. coli* were isolated from children 5 – 8 years of age with diarrhea at school in London. Workers found that these strains invade the epithelial cells and cause diarrhea.

The pathogenicty of *E. coli* was firstly suggested by laurelle in 1889. In the past three types of *E. coli* were recognized Enterotoxigenic *E. coli*, Enteropathogenic *E. coli*, Enteroinvasive *E. coli* (Lambert, 1979).

At present eight types of *E. coli* are recognized Enterotoxigenic *E. coli*, Enteropathogenic *E. coli*, Enteroinvasive *E. coli*, Enteroaggregative *E. coli*, Diffuselly adhering *E. coli*, Uropathogenic *E. coli*, Enterohaemorrhagic *E. coli* and *E. coli* that causes sepsis and meningitis .

1.2 Classification of Escherichia

The genus Escherichia belongs to family enterobacteriaceae (Barrow and Feltham, 1993).

The enterobacteriaceae includes the following tribes:

1- Eschericheae.
2- Klebsielleae.
3- Proteusae.
4- Yersinieae.
5- Erwineae.

The tribe Eschericheae includes five genera:

1- Escherichia.
2- Edwardsiella.
3- Citrobacter.
4- Salmonella.
5- Shiglla.

The genus includes the following species:

1- *E.coli* :

Like many other enterobacteria contains numerous serotypes some of which are associated with certain infections in man and animals, some are particularly associated with diarrheal disease while others causes avareity of extra intestinal infections (Orskov and Orskov,1976).

2- *E.adecarboxylata*:

It was described by Leclerc (1962), stranes were isolated from clinical specimens.

3- *E.fergusonii* :

It was proposed by Farmer *et al*. (1985). It was isolated from animal and human clinical materials.

4- *E.hermanii* :

It was isolated particularly from wounds. It was described by Brenner *et al*. (1982).

5- *E.blattae* :

It was isolated from the intestinal tract of cockroaches and has not been reported in clinical material (Barrow and Feltham, 1993).

6- *E.vulneris* :

It was also described by Brenner *et al*. (1982) as anew species to include group of strains, many of which were isolated from human wounds.

1.3 Definition of *E.coli*

Escherichia coli are a straight Gram negative rod and nonsporing rod. It grows readily on simple nutrient media, occurring singly or in pairs. Most of the organisms are motile with peritrichus flagella. The total number of serotypes is very high (Bergey's Manual of Systematic Bacteriology, 1984).

The organism is a normal inhabitant of the lower part of the intestinal tract of all warm-blooded animals. Usually t is not found in the intestines of fish or other cold-blooded animals. A few numbers or none are found in the stomach and anterior portion of the bowel. It is found in greater abundance in carnivores and omnivores than herbivores (Gillespie and Timoney, 1981). Being a primary components of faeces, it is therefore one of the most ubiquitous bacteria on the surface of the earth (Sojka, 1965). Frequently there are very few numbers of the bacteria in the faeces of cows and horses. Most are harmless saprophytes but others are virulent pathogens that affect the intestine and extra intestinal sites. The major diseases caused by *E.coli* are enteric infections, septicemia, urinary tract infection, and mastitis. Under certain condition the numbers of these organisms undergo a marked and rapid increase *in vivo*, and this may be associated with definite signs of illness and some times death (Buxton and Frazer, 1977). It is aerobic and facultatively anaerobic.

1.4 Normal habitat of *E.coli*

E.coli is a world wide in distribution. Many *E.coli* are part of normal flora of the intestinal tract of human and animal. Some species are free living occurring in soil, water and vegetation (Carter, 1985). *E.coli* becomes established in the intestine shortly after birth when the sterile intestine of the fetus is seeded with bacteria derived from the mother and the environment. *E.coli* passes easily and reaches the intestine because in newborn animal and human the stomach pH is nearly neutral. *E.coli* continues throughout adult live as the intestine and is usually the dominant isolate on aerobic culture of feaces or intestinal contents. Most strains of *E.coli* are harmless commensals

but others are virulent pathogens that affect intestine or extra- intestinal sites (Gyles, 1993).

1.5 Characteristics of *E.coli*

1.5.1 Morphology

Escherichia coli is straight rods measuring 1.1-1.5 by 2.0-6.0µm (living) or 0.4 - 0.7 by 1.0 - 3.0 µm (dried and stained) with parallel sides and rounded ends. It is a Gram-negative rod, that may form chains under unfavorable condition (exposure to penicillin) capsules or microcapsules are produced by many stains. The organism is non acid fast and non – spore former (Jon, 1984).

Many strains possess peritrichous flagella but may be sluggish motile, some strains are non-motile or only feebly motile. The organism stain Gram-negative (Buxton and Frazer, 1977).

1.5.2 Capsules

Certain strains of *E.coli* that cause diarrhea in calves and a subset of strains that cause diarrhea in pigs produce abundant capsular polysaccharide that may aid in colonization of the intestine (Hadad and Gyles, 1982). The polysaccharide capsule is produced *in vivo* and appears to be a virulence factor in these strains. Spontaneous a capsular mutant of these strains failed to colonize the intestine and to produce diarrhea in experimental infected calves (Hadad and Gyles, 1982). It is uncertain, however, whether the acapsular mutants were deficient in structures and/or products other than capsular polysaccharide. Studies on the ultra structure of the capsulated *E.coli* in association with the intestine of calves suggest that the bacteria attached to the intestinal epithelium (Hadad and Gyles, 1982; Acres, 1985).

1.5.3 Cultural characteristics

The organism is aerobic and facultative an aerobic in the presence of a fermentable carbohydrate. Growth occur between 14-45ºc (optimum temperature is 37ºc). Optimum pH for growth is 7 but growth occurs within a wide pH range. It grows readily on ordinary laboratory media. Uniform clouding is produce in broth after 12-18 hours incubation. Colonies on nutrient agar have slightly raised surfaces. Pigments are not produced. Growth on agar slants is in confluent with a turbid water of syneresis. Wide zones of beta type of haemolysis around colonies are produced by some strains. Colonies on agar medium are usually 2-3 mm in diameter (Bale *et al*., 1984).

1.6 Isolation and cultural characterization of *E.coli*

1.6.1 Media for isolation of *E.coli*

Three types of media can be used for primary isolation of *E.coli*:

1.6.1.1 Differential or selective media

A- MacConkey's agar media

It is used to detect coli form and enteric pathogens from faecal samples based on their ability to ferment lactose. Lactose fermenting bacterial species like *E.coli* gives pink to red colinies while other non lactose fermenting organisms give coluorless to transparent colonies after an overnight incubation at 37ºc on this medium.

B- Eosin and methylen blue medium (EMB)

It is used for the isolation of lactose fermenting Gram negative organism like *E.coli*. Eosin and, methylen blue medium consist of peptone base with lactose, sucrose, eosin and methylen blue. Eosin and methylen blue serve as indicators for fermentation as well as inhibiting gram-positive organisms.

On EMB media *E.coli* like lactose fermenting organisms produces a black precipitate. Colonies will be either black or posses dark center with transparent colourless peripheries after an overnight incubation at 37ºc.

1.6.1.2 Enrich medium

Blood agar is used for first isolation for *E.coli* from systemic infection. Blood agar is constituted of tryptose, sodium chloride, heart infusion, agar and 5% sheep blood.

E.coli is an aerobe and facultative anaerobe. On blood agar *E.coli* produce (1-4) mm in diameter colonies after an overnight incubation at 37ºc. The colonies may appear mucoid and some strains are haemolytic due to production of haemolysin.

1.6.1.3 Basic media

Nutrient agar is used for sub culturing of *E.coli* from differential selective or storage media(slant) prior to perform biochemical and serological identification(Monica, 2000).

1.6.1.4 Maintenance and preservation of *E.coli*

E.coli can survive well in holding media as modified Carey-Blair medium for several weeks to month without losing its plasmids (Sack, 1981).

Storage of strains in liquid broth media supplemented with 15% glycerol as cryopreservative at -70ºc gives good stability of the enterotoxin properties as well as of the surface adhesion. Storage of strains on Dorset egg medium at 4ºc is a good alternative for liquid broth media. Lypholization of *E.coli* strains also give a good stability of plasmid for years (Sack, 1981).

1.7 Resistance to physical and chemical agents

E.coli is relatively susceptible to physical and chemical agents. In the majority of instances a temperature of 55ºc for one hour or 60ºc for 20 minutes is lethal to these organisms. They are killed rapidly by autoclaving at 120 º c.Under natural conditions; *E.coli* may survive for weeks or months in water, faeces and dust in animal houses. They are highly susceptible to the lethal action of phenol and cresol, but the efficacy of these disinfectants is reduced in the presence of mucus and faeces (Buxon and Frazer, 1977).

1.8 Biochemical tests

Biochemical tests for the differentiation of *E.coli* from other closely related bacterial groups must be based on the reactions which occur in a variety of media. All strains of *E.coli* ferment glucose and lactose with the production of acid and gas (Buxton and Frazer, 1977), but few strains are late lactose fermenters or may often fail to ferment this sugar (Sojka, 1965). Buxton and Frazer (1977) stated that the majority of strains ferment mannitol, form indole but fail to produce H2S and do not grow on citrate medium, and it dose not produce gelatinase enzyme, Most strains do not develop urease, give a negative voges-proskaur reaction and are positive to the methyl red test.

Milk is coagulated and acidified. Faecal *E.coli* is able to grow in MacConkey's lactose bile broth at 44ºc (Ejkman's test) with the production of gas. This test is of value for water bacteriologists in presumptive identification of faecal *E.coli*. Generally, there is no single biochemical feature which is particularly characteristic of Escherichia group. A comparison of various reactions is required for its classification (Robertson and Maclowry, 1974).

1.9 Antigens and toxins

The complex O, H and K antigenic structure of *E.coli* have been studied in detail because they form the basis on which the serotypes can be differentiated one another (Buxon and Frazer, 1977).

1.9.1 O antigens

These are the somatic antigens occurring as part of the bacterial body and composed of a polysaccharide phospholipids protein complex (Buxon and Frazer, 1977).

1.9.2 K antigens

These antigens which occur as envelops or capsules on most strains of *E.coli*, are composed of polysaccharides. The K antigens show different degrees of thermoability which are the basis for subdividing them into L, A and B varieties (Buxon and Frazer, 1977).

1.9.3 H antigens

These are the flagellar antigens which are composed of protein. Passage of the strain through semi-solid medium often results in increased development of H antigen and of motility. (Buxon and Frazer, 1977)

1.9.4 Fimbrial antigens

Fimbriae occur as small filaments situated over the whole surface of a bacterium. Fimbriae are antigenic, and their antigens are not specific for bacterial serotypes or for groups. Repeated sub-culturing of fimbriated bacteria on solid media may result in the loss of fimbriae. Growth in fluid media encourages their development.

Bacteria possessing fimbriae agglutinated red blood cells of various animal species and man. This form of haemagglutination reaction is due to the adherence of fimbriae to the surface of red blood cells (Buxon and Frazer, 1977).

1.9.5 Common antigens (CA)

Some serotypes develop another somatic antigen in addition to the O antigen referred to above. It appears to be common to many members of different bacterial groups, it is composed of polysaccharide (Buxoon and Frazer, 1977).

1.10 Classification of pathogenic *E.coli*

Pathogenic *E.coli* differs from the non-pathogenic *E.coli* by the presence of virulence factors organized in clusters in the chromosome or plasmid. According to the large variation in DNA content and to the difference in the distribution of genomic location (insertion site) of different virulence determinants, pathogenic *E.coli* were divided into eight major categories(Puente and Finlay, 2001).

1.10.1 Entero-toxigenic *E.coli* (ETEC)

ETEC cause watery diarrhea, ranging in severity from mild and self-limiting to severe cholera-like profuse diarrhea (Sack, 1975).

1.10.1.1 Prevalence of the disease caused by ETEC

In human all ages are susceptible to the disease, the disease is less common in breast fed infant. During the weaning the disease is common (Guerrant *et al.*, 1975).In adult the disease usually affects the travelers (Black, 1990).

In calves the disease is restricted to those under 4 months of age (Holland, 1990).

1.10.2 Enteroinvasive *E.coli* (EIEC)

Enteroinvasive *E.coli* (EIEC) were characterized by their positive reaction in the Sereny test, in which strains are tested for their ability to cause keratoconjunctivitis in guinea pig eyes. This is a characteristic which EIEC share with strains of Shigella (Ephros *et al*., 1996).

1.10.2.1 Virulence factors of EIEC

EIEC secretes invasion plasmid antigens (Ipas) A-D for invasion of the host cell (colonic epithelium) (Kocks *et al.*, 1995).

1.10.3 Enteropathogenic *E.coli* (EPEC)

It is the predominant cause of infant diarrhea world wide and affects children under 6 years (Levine and Edelman, 1984).

1.10.3.1 Virulence factors of EPEC

EPEC in order to develop lesion it requires three stages. First stage is characterized by the initial non intimate attachment to epithelial cell surface in a pattern termed localized adherence (L.A) (Giron *et al.*, 1991). During the second stage a set of EPEC secreted proteins (ESPs) leading to a complex response by the epithelial cell, and during the third stage and outer membrane protein called intimin allows EPEC to attach intimately to the host cell membrane on interaction with its translocated intimin receptor called "tir" (Lia *et al.*, 1997).

1.10.4 Enterohaemorrhagic or verotoxigenic *E.coli* (EHEC)

It causes bloody watery diarrhea, abdominal pain, fever and vomiting. In severe cases it leads to heamolytic anemia (Brunder *et al.*, 1997).

1.10.4.1 Virulence factors of EHEC

For EHEI to cause disease three major virulence attribute, the capacity to causes formation of attaching and effacing lesions (A/B) (Brunder, 1999).

1.10.5 Enteroaggregative E.coli (EAEC)

Enteroaggregative adherence demonstrated by enteroaggregative *E.coli* (EAggEC) consists in the bacteria aligning themselves in parallel rows to their tissue cells or glass. These EAggEC are mainly associated with persistent childhood diarrhea in Brazil, Chile, Mexico and India (Cobeljic *et al*., 1996).

1.10.5.1 Virulence factors of EAEC

EAEC strains adhere to cultured cells in small clumps or aggregates through aggregative adherence fimbriae (AAFıand AAFıı). EAEC strains produce two types of enterotoxin (Eslava *et al.,* 1998).

1.10.6 Diffusely adhering *E.coli* (DAEC)

Diffuse-adhering *Escherichia coli* (DAEC) strains were significantly associated with persistent watery diarrhea in children between 2-5 years (Giron *et al*., 1991).

1.10.6.1 Virulence factors of DAEC

DAEC has been associated with four different adhesions while toxins have not been described (Peiffer *et al.*, 1998).

1.10.7 Uropathogenic *E.coli* (UPEC)

It causes urinary tract infections (UTI) (Jose *et al.*, 2002).

1.10.7.1 Virulence factors of UPEC

In order to successfully colonize and establish urinary tract infection, UPEC encode several adhesion, both are fimbrial or non-fimbrial adhesion (afimbrial adhesion Afa-I and Afa-III) (Johnson, 1991).

1.10.8 *E.coli* that causes neonatal sepsis and meningitis

In addition to gasterointestinal and urinary tract infection, some strains of E.coli can cause septic (invasive) diseases in newborn (Quagliarello and Scheld 1992).

1.10.8.1 Virulence factors

E.coli that causes septic diseases produces a polysaccharide capsule that blocks complement and antibody deposition on the bacterial surface and thus avoid uptake and clearance by antibody-mediated immune events (Roins *et al.*, 1974). *E.coli* that causes meningitis and sepsis often produces type-1 pili. (Korhonen *et al.*, 1985).

1.11 Enterotoxin

Enterotoxin is a common cause of diarrhea in animal (Buxton and Frazer, 1977) and infants in developing countries (Jawetz *et al*., 1995). They are divided according to their heat stability and their mechanism of action into: Heat-labile (LT) enterotoxin and Heat-stable (ST) enterotoxin.

1.12 Mode of Transmission

Transmission of *E.coli*through ingestion of contaminated food, water, milk,and vegetables. Infection by *E.coli* require large dose 10 9 cfu of bacteria to be ingested or inoculated (Lambert, 1979).

1.13 Epidemiology and pathology

Pathogenic strains of *E.coli* are associated with diseases of the intestine, septicemias of newborn or young animals and with respiratory tract disease of poultry. The disease is characteristically found in newborn or young animals and a variety of epidemiological factors can be involved. Moreover etiological agents other than *E.coli* can be present at the same time. A critical factor is the immune status of the newborn animals.

ETEC is an under recognized but extremely important cause of diarrhea in the developing world where there is inadequate clean water and poor sanitation and also the most common cause of traveler's diarrhea. ETEC diarrhea is most frequently seen in children, suggesting that a protective immune response occurs with age (Qadri *et al*., 2005).

1.14 *E.coli* infection in animals and man

The organism produce colibacillosis in all species of newborn farm animals, it is a major cause of loss in this age group (Blood *et al.*, 1990). Infection also occurs in man and poultry with various maninfestation.

1.14.1 Cattle

1.14.1.1 Colibacillosis

Diarrhea in newborn calves under 10 days of age is one of the most common diseases. It may cause intestinal and extra intestinal infections (Salvadori *et al.*, 2003). It is a major cause of economical losses in cattle herds. The treatment and control of neonatal diarrhea in calves have been difficult.There are three different types of the disease.

I enteric colibacillosis

This is manifested mainly by varying degrees of diarrhea.

II septicemic colibacillosis

This is manifested by septicemia and rapid death. Occur only in very young calves. Colostrum provides protection against coli septicemia in calves but does not prevent diarrhea.

III Enterotoxic colibacillosis

Enterotoxic colibacillosis characterized byenteric toxemia. Diarrhea is usually not evident in spite of the slightly distended abdomen (Blood *et al.*, 1990).

All breeds of beet and diary calves are affected. Out breaks are more common in case of intensive livestock rearing. In small herds, the disease occurs only sporadically during calving season (Gillespe and Timoney, 1981). Isolation of the organism from the internal organs of an aborted bovine fetus was reported by Moorthy (1985).

1.14.1.2 Mastitis

Bovine mastitis due to *E.coli*, Klebsiella, or Enterobacter is referred to as coliform mastitis and may occur as a peracute, acute, chronic, or subclinical infection (Nemeth, 1992). *E.coli* is by for the most important of the Gram negative environmental organisms that cause mastitis in diary

cattle. Anonymous 1977 reported mastitis after inoculation of *E.coli* into the mammary gland of healthy cows. Bacteria in the environment invade via the teat canal and establish a local infection that is confined to the udder.

No markers have been identified to distinguish strains of *E.coli* that cause mastitis from strains in normal faeces and the environment. *E.coli* recovered from the udders of cattle with mastitis belong to a wide range of O serogroups and exhibit biochemical properties indistinguishable from those of *E.coli* from the faeces and environment (Nemeth, 1987). Although capsule and serum resistance have been suggested to be virulence factors for mastitic *E.coli*, those *E.coli* that cause bovine mastitis appear to be simply opportunistic environmental organisms that have no special virulence factors.

The antibacterial proprieties of milk, the un availability of free iron in milk, and the levels of specific opsonins in milk undoubtedly function in defense against infection in the mammary gland. Polymorph nuclear leukocytes constitute the major antibacterial defense against bacteria invading the mammary gland and effectively phagocytes and destroy bacteria opsonized by IgM (Nemeth, 1987) has been implicated in necrosis of the superficial layer.

1.14.2 Sheep

Some cases of colibacilosis in lambs develop enteric signs but the majority of cases are septicemic and per acute. Tow age groups are affected lambs 1-2 days old and lambs 3-8 weeks old. Per acute cases are found dead without showing any signs. Acute cases are characterized by collapse, occasionally signs of meningitis, followed by recumbency (Blood *et al*., 1990).

Lambs with enteric colibacillosis develop diarrhea, depression which may be followed by death. Less severe symptoms include meningitis and arthritis (Gillespie and Timoney, 1981). Abortion attributed to *E.coli* infection was reported by Howarth (1932).

1.14.3 Goats

Muller (1960) reproduced sterility in female goats when served by males infected with *E.coli* haemolytic strains. The organism was the only pathogen recovered from 34 diarrheic goats by rectal swabs over a period of one year in India. Serogroups O24, O28, O45, O54, O61, O121, O138, and O148 were identified (Osmani *et al.*, 1992).

1.15.4 Camels

An endemic *E.coli* infection in new born camels was reported by Romboli (1942). It resembled neonatal septicaemia in other farm animals. The most common manifestation of the intestinal form of the disease was diarrhea. Chauhan and Kaushik (1991) isolated enterotoxigenic *E.coli* from camels with diarrhea.

1.14.5 Horses

Buxom and Frazer (1977) suggested that newborn foals can suffer from a disease which may be associated with a variety of bacteria including *E.coli*, abortion and metritis had been reported in mares (Dimock *et al.*, 1947).

1.14.6 Pigs

Hagan and Burner (1988) mentioned that distinct manifestations of enteric colibacillosis were seen in swine include neonatel *E.coli* enteritis, enteritis shortly after wearning and odema disease in various body tissue of pigs soon after weaning. *E.coli* also had been shown to be a cause of acute meningitis and fibrinous poly serositis in piglets (wilkie, 1981).

1.14.7 Dogs

E.coli, particularly the haemolytic strains, have been associated with acute generalized infections in puppies and with either intestinal or urogenital infections in older animals including pyometra and occasionally cystitis (Buxton and Frazer, 1977). Haemorrhagic gastro-enteritis was reported in puppies up to 4 weeks of age. The disease was characterized by haemorrhagic gastro-enteritis, congestion of the lungs and hearts. Meningitis and haemorrhages covering the whole carcasses were also observed (Mansi, 1962).

Sinior *et al*. (1992) recovered 82 *E.coli* isolates from dogs with urinary tract infections. E.coli is the most frequent cause of (UTI) urinary tract infection in dogs and cats (Oxendford *et al.*, 1984).

1.14.8 Cats

Experimental *E.coli* infection was reported in 5-8 months old cats by IIIyutovich *et al*. (1962). The cats were fed orally *E.coli* serotype O111:H12. Enteritis was developed in 2-6 days after administration. The test organism was isolated on the second day after dosing and continued to be excreted in the faeces until the 57^{th} day.

1.14.9 Avian pathogenic *E.coli* (APEC)

E.coli adversely affects avian species through infection of the blood, respiratory tract, and soft tissues. Disease resulting from *E.coli* infections, such as colibacillosis air sacculitis and cellulites, cause high morbidity and mortality in poultry, which have a significant economic impact on the poultry industry (Bass *et al*.,1999). Avian colibacillosis is complex syndrome characterized by multiple organ lesions with air sacculitis and associated pericarditis, perihepatitis and peritonitis being most typical. More

than 20(APEC) strains have been investigated in animal experiments (Ewers *et al*., 2003).

Like most pathogenic *E.coli*, avian isolates can not be distinguished biochemically from the normal common sales inhibiting the gastrointestinal tract of birds. Using a molecular approach we were able to identify genetic difference among avian *E.coli* isolates by restriction fragment length poly morphism (RFLP) and random amplification of poly morphic DNA (RAPD) by the polymerase chain reaction (PCR) (Maurer *et al*., 1998).

Cellulites derived strains presented phenotypic and genotypic characteristic of greater virulence than did the fecal isolates from healthy chickens (De Brito *et al*., 2003).

The organism causes the following disease when it spread via the circulatory system:

1.14.9.1 Acute septicemia

Synonyms coli septicaemia, colibacillosis, *E.coli* infection, septic pericarditis, and air sacculitis (Sokia and Carnaghan.1961).This disease is a major threat to broiler production with an increasing incidence due to increased intensive production. Growing chickens and turkeys are affected. Broiler chickens 6-10 weeks old are being more susceptible. The most common serotypes causing the disease are O1: KI (L), O2:K1 (L) and O78: k80 (B) (Hostad *et al*., 1978).

1.15.9.2 Embryo and early chick mortality

In chicks up to 3 weeks of age, the yolk sac is the predilection site of infection (Gordon and Jordan, 1982). Infections occurs as a result of contamination of eggs, whether from faeces or from infection of the ovary of the hen. Affected chicks develop omphalitis and mushy-yolk disease

(Cheville and Arp, 1978). The condition was reproduced following air-sac injection with *E.coli* serogroup O78 (Sasipreeyajan and Pakpinyo, 1992).

1.14.9.3 Respiratory tract infection (air sac disease)

Five to twelve weeks old chickens and particularly broilers are affected. Inhalation of contaminated dust is the main route of transmission. The air sacs are thickened and there is often a caseous exudates on the respiratory surfaces (Cheville and Arp, 1978).

1.14.9.4 Synovitis

Young birds of all species are affected. Knee, elbow, shoulder, hock and foot joints are affected mostly. They may be thickened and swollen causing severe lameness. The organism may spread to other organs including kidneys, pectoral muscles and bone marrow (Gordon and Jordan, 1982).

1.14.9.5 Panophthalmitis

Usually one eye is affected. It becomes blind as a result of hypopyon and destruction of the retina (Hofestad *et al.*, 1978).

1.14.9.6 Coligranuloma (Hjarre's disease)

This is characterized by coli granuloma of Turkeys. The spleen may be affected. Well developed lesion resembles a tubercular granuloma (Hoftad *et al*., 1978).

1.14.9.7 Enteritis

Affected birds exhibit weakness, inappetance, diarrhea and cyanosis of the wattles. Lesions include petechial hemorrhagic in the intestines, heart, lungs and kidneys. The liver is enlarged (Nagi and Khana, 1967).

1.14.9.8 Pericarditis

Pericardial tissue appears to be the predilection site for septicaemic *E.coli* strains. Pericaditis may be associated with perihepatitis (Borstein and Samberg, 1953) omphalitis or respiratory disease.

1.14.10 Human

1.14.10.1 Childhood Diarrhea

Diarrhea is a leading cause of morbidity and mortality among children in developing countries. The bacterial pathogen most commonly associated with endemic forms of childhood diarrhea is *Escherichia coli*. At least six categories of diarrheagenic *E.coli* have been described: enteropathogenic *E.coli* (EPEC), enterotoxigenic *E.coli* (ETEC), enteroinvasive *E.coli* (EIEC), enterohemorrhagic *E.coli* (EHEC), enteroaggregative *E.coli* (EAEC) and diffusely adherent *E.coli* (DAEC). Two additional categories cell detaching *E.coli* (CDEC) and cytolethal distending toxin producing *E.coli* (CLDTEC), have been proposed. The epidemiological significance of each *E.coli* category in childhood varies with geographical area.

Epidemiological evidence and human challenge studies have demonstrated unequivocally that EPEC, ETEC, EIEC and EHEC are important causes of diarrhea world wide (Okeke *et al*., 2000).

1.14.10.2 *E.coli* O157:H7

E.coli o157:H7 is a common cause of a variety of illnesses including bloody diarrhea and the hemolytic uremic syndrome (HUS). The O157:H7 serotype was first described in the literature in 1983 following two outbreaks of hemorrhagic colitis in a fast-food restaurant chain in Organ and Michigan in 1982. The most frequent mode of transmission for *E.coli* O157:H7infections is through consumption of contaminated food and water, and several outbreaks have been caused by ground beef. Approximately 1%

of healthy cattle may have the organism in their intestines (Arne lindsted *et al*., 2003).

1.14.10.3 Urinary tract infection (UTI)

Urinary tract infections are common clinical entities occurring in a variety of patient groups, most frequently caused by uropathogenic *E.coli* (Stapleton, 2003).

1.15 Antibiotics

1.15.1 Definition

An organism protects itself from enemies in various ways. It may produce metabolic waste products, which change the condition in medium, such as pH, osmotic pressure and surface tension making the environment unfavorable to the growth of less tolerant organisms. It may elaborate specific toxic substances, which interfere with the metabolism of other organism to such an extent that they are either killed or prevented from multiplying. These specific toxic substance are called antibiotics (Salle, 1971). Heritage *et al*. (1996) defined an Antibiotic as a substance that was produced by microorganism that in very low concentration inhibits or kills the growth of another microorganism.

1.15.2 Classification and mechanism of action

Since antibiotics were first discovered in the 1920s, much knowledge has been acquired on their mode of action and the significance of this action on their relative merits in the therapy of man and animals.

Antibacterial agents can be divided into four groups as they affect the synthesis of nucleic acid, protein, the formation of the cell wall and permeability of cell membrane.

1.15.2.1 Nucleic acid inhibitors

Replication of the nucleic acids of the bacterial cell is prevented directly by nalidixic acid and rifamycin and indirectly by the sulphonamides. Sulphonamides ultimately deprive the cell of nucleic acid and the presence of nalidixic acid prevents its replication.

Sulphonamides are structural analogues of para-amino benzoic acid PABA and competitively inhibit an enzymatic step (dihydropteroate synthase) during which PABA in incorporate in the synthesis is reduced, the levels of tetrahydropholate (folinic acid). Because dihydropteroate synthesis is reduced, the levels of tetradihydropteroate (folinic acid) formed from the dihydropteroate diminishes. Tetrahydropholate is an essential component of the coenzymes responsible for single carbon metabolism in cells. Acting as antimetabolites to PABA, sulphonamides eventually block, in a complex fashion, several enzymes. These enzymes include those needed for the biogenesis of methionine, glycine and formymethionyl-transfer RNA. These results in suppression of protein synthesis impairment of metabolic processes and inhibition of growth and multiplication of those organisms that can not use preformed folate. The effect is bacteriostatic, although a bactericidal action is evident at high concentration that may occur in urine (Aiello and Mays, 1998).

Quinolones inhibited bacterial DNA gyrases (Topoisomerases) which was catalyzed supercoiling of bacterial DNA (Thomas, 1993).

Nalidixic acid inhibits DNA synthesis without affecting RNA synthesis and is employed mainly for urinary infections caused by Gram-negative bacteria (Carter, 1985).

1.15.2.2 Protein synthesis inhibitors

Antibiotic classes that act by inhibiting protein synthesis include aminoglycoside e.g. gentamicin, tobramycin, kanamycin and streptomycin, tetracycline, chloramphenicol, macrolides, e.g erythromycin and azithromycin, and lincosamides, e.g clindamycin (Forbes *et al*., 1998; Cheesbrough, 2000).

Aminoglycosides inhibit bacterial protein synthesis by binding to protein receptors on the organism's 30S ribosomal subunit. This process interrupts several steps, including initial formation of the protein synthesis complex, accurate reading of the mRNA code, and disruption of the ribosomal- mRNA complex (Baker and Breach, 1980; Forbes *et al*., 1998).

Macrolides (erythromycin and azithromycin) bind to the 50S subunit of the ribosome and the binding site is a 23S rRNA (Jawetz *et al*., 1998).

Chloramphenicol inhibits the addition of new amino acids to the growing peptide chain by binding to the 50S ribosomal subunit (Jawetz *et al*., 1998). This antibiotic is highly active against a variety of Gram-negative and Gram- positive bacteria (Forbes *et al*., 1998).

Tetracyclines inhibit protein synthesis by binding to the 30S ribosomal subunit so that incoming tRNA- amino acid complexes cannot bind to the ribosome, thus halting peptide chain elongated. Tetracyclines have a broad spectrum of activity that includes Gram- negative bacteria, Gram- positive bacteria, and several intracellular bacterial pathogens such as Chlamydia and rickettsia (Jawetz *et al*., 1989;Forbes *et al*.,1998).

Lincosamides (clindamycin) inhibited protein synthesis by binding to receptors on the bacterial 50S ribosomal subunit and subsequent disruption of the growing peptide chain. Primarily because of uptake difficulties associated with Gram- negative outer membrane, the macrolides and

clindamycin generally are not effective against most genera of Gram-negative bacteria. However, they are effective against Gram- positive bacteria (Forbes *et al*., 1998).

1.15.2.3 Cell wall synthesis inhibitors

The bacterial cell wall known as the peptidoglycan, or murein layer, plays an essential role in the life of the bacterial cell. The cell wall of bacteria is tough and rigid and lies external to the cell membrane, giving the whole cell protection from possible osmotic damage (Forbes *et al*., 1998). Several agents affect cell wall synthesis, the most important being penicillins e.g. cloxacillin, ampicillin and amoxicillin, cephalosporins e.g. cephradine, cefuroxime and ceftazidime and glycopeptide e.g. vancomycin (Cheesbrough, 2000).

Beta- lactam antimicrobial agents are those that contain the four-membered, nitrogen- containing, beta lactam ring at the core of their structure, and mode of action of these drugs that target and inhibit cell wall synthesis by binding the enzymes involved in synthesis. The beta-lactam antibiotics inhibit the last step in peptidoglycan synthesis, the final crosslinking between peptide side chain, mediated by bacterial carboxypeptidase and transpeptidase enzymes (Forbes *et al*., 1998).

Glycopeptides which are the other major class of antibiotic that inhibit bacterial cell wall synthesis, e.g. vancomycin is the most commonly used agent in this class inhibits cell wall synthesis by binding to precursors of cell wall synthesis (Forbes *et al*., 1998). Vancomycin can not penetrate the outer membrane of most Gram- negative bacteria to reach their cell wall precursor targets, because of its relatively large size. There fore, this agent is usually ineffective against Gram- negative bacteria (Forbes *et al*., 1998).

Some fungi of *cephalosporium spp* yield antimicrobial substances called cephalosporins (Jawetz *et al*., 1989). Cephalosporins consisted of three generations first one was cephradine and cephalothin, second was cefuroxime and third were ceftazidime and cefotaxime ((Jawetz *et al*., 1989; Cheesbrough, 2000).

The mechanism of action of cephalosporins is analogous to that of penicillins by binding to specific PBPs that serve as drug receptors on bacteria; inhibiting cell wall synthesis by blocking the transpeptidation of peptidoglycan; and activating autolytic enzymes in the cell wall that can produce lesions resulting in bacterial death (Jawetz *et al*., 1989).

1.15.2.4 Cell membrane function inhibitors

Polymyxins (polymyxin B and colistin) are the agents most commonly used that disrupt bacterial cell membranes. Most notably, they are more active against Gram- negative bacteria, while activity against Gram- positive bacteria tends to be poor (Forbes *et al*., 1998). Polymyxin becomes firmly bound to the cytoplasmic membrane and acts by damaging this structure (Thomas, 1993).

1.15.3 Toxicity and side effects

Not all antimicrobial, at the concentration required to be effective are completely non- toxic to human cells. Most, however, show sufficient selective toxicity to be of value in the treatment of microbial disease (Cheesbrough, 2000). Most serious side effects of penicillin are due to hypersensitivity. The tetracycline and chloramphenicol produce varyng degrees of gastrointestinal upset (nausea, vomiting, and diarrhea).

Gentamicin was toxic, particulary in the presence of impaired renal function. Fever, skin rashes and other allergic manifestation may result from hypersensitivity to streptomycin (Jawetz *et al*., 1989).

1.15.4 Antibiotic spectrum

1.15.4.1 Broad spectrum antibiotics

The term broad spectrum is applied to antibacterial agents with activity against a wide range of Gram- positive and Gram- negative organisms such as tetracyclines, aminoglycosides, sulphonamides and chloramphenicol (Thomas, 1993; Cheesbrough, 2000).

1.15.4.2 Narrow spectrum antibiotics

Narrow spectrum antibiotics are those with activity against one or few types of bacteria, e.g. vancomycin against staphylococci and enterococci (Cheesbrough, 2000).

1.15.5 Type of action

Antimicrobial agents are generally described as bacteristatic when, at usual dosage, they prevent the active multiplication of bacteria, e.g. chloramphenicol, tetracycline and erythromycin, and are described as bactericidal when, at usual dosage, they kill bacteria, e.g. the penicillins, cephalosporins, glycopeptides and aminoglycosides (Cheesbrough, 2000).

Some bacteristatic agents become bactericidal when used at higher concentration e.g. erythromycin and tetracycline (Thomas, 1993; Cheesbrough, 2000).

1.15.6 Clinical use of antibiotics

The object of antibiotic therapy is to cure the patient with the minimum of complications and discomfort. At the same time, it is important to discourage the emergence of drug- resistant organisms. The principles should observed are, antibiotics should not be given for trivial infections they should be used for prophylaxis only in special circumstances, treatment should be based on a clear clinical and bacteriological diagnosis, the choice of antibiotic is essentially a clinical matter. Antibiotics for systemic

treatment should be given in full therapeutic doses for an adequate period. In local treatment of superficial infections it is important to use antiseptics which are rarely or never used systematically, e.g. mupirocin, bacitracin and polymyxin. Antibiotic solutions and powders should not be liberated into the environment. They can cause hypersensitivity reactions and encourage development of antibiotic- resistant strains (Thomas, 1993).

1.16 Drug Resistant

If bacteria are repeatedly subcultured in the presence of gradually increasing subinhibitory concentration of an antibiotic it is usually possible to obtain mutant organisms which will survive and multiply in concentration which are lethal for the parent strain (Thomas, 1993).

Antibiotics can be inactivated either by enzymatic cleavage or by chemical modification such that they no longer interact with the target site or are no longer taken up by the organism rendering them inactivates (Pratt, 1990; Lancini, 1995).

The development of antibiotic- resistant strains during therapy was unlikely to be a serious clinical problem because the fraction of resistant cells in bacterial population was always very small (Davies, 1994).

Drug resistant has increased substantially in recent years and has reduced the value of formerly widely prescribed agents such as the sulphonamides and ampicillin (Hugo and Russell, 1989).

1.16.1 Multidrug resistance

Often bacterial isolates are multidrug resistant; however the vast majority of studies have looked at the detection and diagnosis of resistance caused by one class of antimicrobial agents while others have looked at the detection of resistance to multiple antimicrobial agents (Fluit *et al*., 2001). Resistant enterobacteriaceae frequently contain multiple plasmids, the larger

of which can carry genes for resistance to 10 or more antimicrobial agents (Jacoby, 1991). These highly resistant bacteria were made many currently available antimicrobial drugs ineffective and in certain instances were already posing important public health problem (Cohen, 1992).

Integrons are a special case of multidrug resistance. Integrons are genetic elements that contain the genetic determinant of a site-specific recombination system that recognize and captures mobile gene cassettes. An integron contains an integrase and an adjacent recombination site. Gene cassettes can be integrated by the integrase at the recombination site, and multiple gene cassettes can be present in one integron (Fluit *et al*., 2001). Four classes of integron have been described. Class I integrons are often associated with the sulfonamide resistance gene (sull) and are the most common integrons. Calss 2 integrons are associated with Tn 7.Only one class 3 integron has been described, and class 4 is limited to *Vibrio chlderae.* Integrons are found almost exlusively in Gram-negative bacteria, with one known exception. At least 60 gene cassettes have been described for class I integrons. The majority of genes encode antibiotic and disinfectant resistance, including resistance to amino glycosides penicillins, cephalosporins, trimethoprim, tetracycline, erythromycin and chloramphenicol (Fluit *et al*., 2001).

1.16.2 Mechanisms of antibiotics resistance

Successful bacterial resistance to antimicrobial action requires interruption or disturbance of one or more of the steps essential for effective antimicrobial action. These disturbance or resistance mechanisms can come about in various ways, but the end result is partial or complete loss of antibiotic effectiveness. Different aspects concerning antimicrobial resistance mechanisms include biologic vs. clinical antimicrobial resistance,

environmentally mediated antimicrobial resistance, and microorganism-mediated antimicrobial resistance (Forbes *et al*., 1998).

1.16.2.1 Biologic vs. clinical resistance

Development of bacterial resistance to antimicrobial agents to which they were originally susceptible requires alterations the cell's physiology or structure. Biologic resistance refers to changes that result in the organism being less susceptible to a particular antimicrobial agent than has been previously observed. When antimicrobial susceptibility has been lost to such an extent that the drug is no longer effective for clinical use the organism has achieved clinical resistance (Forbes *et al*., 1998).

1.16.2.2 Environmentally mediated antimicrobial resistance

Environmentally mediated resistance is defined as resistance that directly results from physical or chemical characteristics of the environment that either directly alters the antimicrobial agent or alters the microorganism's normal physiologic response to the drug. Examples of environmental factors that mediate resistance includes pH, anaerobic atmosphere, cation (e.g. Mg++ and Ca++) concentrations, and thymine-thymidine content (Forbes *et al*., 1998).

1.16.2.3 Microorganism- mediated antimicrobial resistance

Microorganism- mediated resistance refers to antimicrobial resistance that is due to genetically encoded traits of the microorganism and is the type of resistance that *in vivo* susceptibility testing methods are targeted to detect (Forbes *et al*., 1998). Organism- based resistance can be divided into two subcategories, intrinsic or inherent resistance and acquired resistance.

1.16.2.3.1 Intrinsic resistance

Intrinsic resistance is antimicrobial resistance resulting from the normal genetic, structural, or physiological state of microorganism

(Forbes *et al*., 1998). Such resistance is considered to be natural and consistently inherited characteristic that is associated with the vast majority of strains that constitute a particular bacterial group, genus or species. There for, this is predictable resistance so that once the identity of the organism is known, so are certain aspects of its antimicrobial resistance profile (Forbes *et al*., 1998).

1.16.2.3.2 Acquired resistance

Bacteria can acquired resistance to antibiotics as a result of a chromosomal mutation, expression of a latent chromosomal gene, by exchange of genetic material through transformation (the exchange of DNA), transduction (bacteriophage), or conjugation by plasmids (extrachromosomal DNA) (Neu, 1992).

Acquired resistant was usually reversible. However, the ease with which resistance reverts to sensitivity depends upon a number of factors such as nature of the organism, nature of the drug, degree of resistance that has been established, and whether the resistance has been acquired by genetic or phenotypic adaptation (Salle, 1971). It was antibiotic resistance that results from altered cellular physiology and structure caused by changes in microorganism's usual genetic makeup (Forbes *et al*., 1998).

The basic mechanisms of acquired microbial resistance to antimicrobial agents were generally divided into five categories, the development of an altered drug target; a decrease in the concentration of drug that reaches the receptors by altered rates of entry or removal of the drug; degradation of the antibiotic; synthesis of resistant, or alternate metabolic pathways that were no longer susceptible to an antibiotic; and failure to metabolize the drug to its active state (Neu, 1992; Davies, 1992; Spartt, 1994; Brody, 1994).

1.16.3 Common pathways for antimicrobial resistance

Whether resistance is intrinsic or acquired, bacteria share similar pathways or strategies to affect resistance to antimicrobial agents. The pathways involve enzymatic or alteration of the antibiotic, decreased intracellular uptake or accumulation of drug, and altered antibiotic target are the most common (Davies, 1994; Forbes *et al*., 1998).

Based on their reaction to staining protocol, bacteria were divided into Gram- negative and Gram- positive classifications. Gram- positive bacteria were surrounded by a thick, rigid, porous cell wall composed of peptidoglycans. It offers little resistance to the diffusion of small molecules such as antibiotics. Gram- negative bacteria have an additional outer membrane composed of lipopolysaccharide that was located around the cytoplasmic membrane and the thin peptidoglycan layer (Bordy, 1994).

1.16.3.1 Resistance to β-lactam antibiotic

β-lactam antibiotics are among the most commonly used antimicrobial agents. They act on penicillin binding proteins (PBPs), which are involved in cell wall synthesis. Penicillin, a β-lactam antibiotic, was one of the first antibiotics. Resistance is most often caused by the presence of β-lactamases, but mutations in PBPs resulting in reduced affinity for β-lactam antibiotics are also commonly observed. Resistance is less frequently caused by reduced uptake due to changes in the cell wall or active efflux. Genes encoding β-lactamases can locate either on plasmids or the bacterial chromosome and are found among both Gram-negative and Gram-positive organisms (Fluit *et al*., 2001).

1.16.3.2 Resistance to aminoglycosides

Aminoglycosides such as gentamicin, tobramycin, amikacin and streptomycin are commonly used antimicrobial agents in the treatment of

infection by both Gram-negative and Gram-positive organisms. Amino glycosides bind to the ribosome and thus interfere with protein synthesis. Resistance to these antimicrobial agents is widespread, with more than 50 amino glycoside-modifying enzymes already described. Most of these genes are associated with Gram-negative bacteria. Depending on their type of modification, these enzymes are classified as aminoglycoside acetyltransferases (AAC), amino glycoside adenyltransferases, also named amino glycoside nucleotidyltransferases (ANT), and amino glycoside phosphotransferases (APH). Amino glycosides modified at amino groups by (AAC) enzymes or at hydroxyl groups by (ANT) or (AOH) enzymes lose their ribosome-binding ability and thus no longer inhibit protein synthesis (Fluit *et al*., 2001).

1.16.3.3 Resistance to fluroquinolones

Fluroquinolones antibiotics exert their antibacterial effects by inhibition of certain bacterial topoisomerase enzymes namely, DNA gyrase (bacterial topoisomerase Π) and (topoisoisomerase IV). These essential bacterial enzymes alter the topology of double-stranded DNA (ds DNA) within the cell. DNA gyrase and topoisomerase IV are heteroterameric proteins composed of two subunits, designated A and B. The genes encoding the A and B subunits are referred to as gyrase A and gyrase B (DNA gyrase) or par C and par E (DNA topoisomerase IV (grl A and grl B in S.aureus)). DNA gyrase is the only enzyme that can effect super coiling of DNA. Inhibition of this activity by fluroquinolones is associated with rapid killing of the bacterial cell. Topoisomerase IV is predominantly responsible for the separation of daughter DNA strands during cell division (Fluit *et al*., 2001).

Mechanisms of bacterial resistance to fluoroquinolones fall into two principal categories, alteration in drug target enzymes and alteration that

limit the permeation of drug to the target. The target enzymes are most commonly altered in domains near the enzyme active site and in some cases reduced drug binding affinity has been demonstrated (Fluit *et al*., 2001).

In Gram-negative organisms, DNA gyrase seems to be the primary target for all quinolones. In Gram-positive organisms, topoisomerase IV or DNA gyrase is the primary target depending on the fluoroquinolone considered the quinolone structure determines the mode of antibacterial action. The primary target seems to depend on the bacterial species as well as on the quinolone structure (Fluit *et al*., 2001).

1.16.3.4 Resistance to glycopeptides

Vancomycin and teicoplanin are glycopeptide antibiotics of clinical interest. The antimicrobial activity is due to binding to D-alanyl –D-alanine side chains of peptidoglycan or its precursors thereby preventing cross-linking of the peptidoglycan chain. Antimicrobial activity of glycopeptide antibiotics is largely limited to Gram-negative bacteria inside the outer cell membrane can not be reached by the glycol peptide molecule. Not all Gram-positive organisms are susceptible to the glycol peptide antimicrobial agents (Fluit *et al*., 2001).

1.16.3.5 Resistance to tetracyclines

Tetracyclines probably penetrate bacterial cells by passive diffusion. Tetracycline act by binding to the 30S ribosomal subunit, resulting in the inhibition of protein synthesis. A growing number of bacterial species have acquired resistance to the bacteriostatic activity of tetracycline. At least 24 tetracycline resistance (Tet) determinants and three oxytetracycline resistance (Otr) determinant (Fluit *et al*., 2001).

Most of the resistance genes code for one of the two important mechanisms of tetracycline resistance, either efflux or ribosomal protection.

These two widespread mechanisms of bacterial resistance to tetracycline do not destroy the compound. Efflux is mediated by energy-dependent efflux pumps, the other important mechanism involves and elongation factor G-like protein that confers ribosome protection. Oxidative destruction of tetracycline has been found in a few species (Fluit *et al*., 2001).

1.16.3.6 Resistance to trimethoprim

Trimethoprin is an analog of dihydrofolic acid, an essential component in the synthesis of amino acid and nucleotides that competitively inhibits the enzyme dihydrofolate reductase (DHFR). Resistance can be caused by a number of mechanisms including overproduction of the host DHFR, mutation in the structural gene for DHFR, and the acquisition of gene (dfr) encoding a resistant DHFR enzyme. The latter mechanism is the most important in clinical isolates (Fluit *et al*., 2001).

At least 15 DHFR enzyme types are known based on their properties and sequence homology (Fluit *et al*., 2001). On of the first studies which described the use of molecular techniques for the detection of trimethoprim resistance investigated the presence of type I and type Π DHFR in diverse Gram-negative isolates showing high levels of trimethoprim resistance (Fluit *et al*., 2001).

1.16.3.7 Resistance to chloramphenicol

Chloramphenicol binds to the 50s ribosomal subunit and inhibits to the peptidyl transferase step in protein synthesis (Fluit *et al*., 2001). Resistance to chloramphenicol is generally due to inactivation of the antibiotic by a chloramphenicol acetyltransferase. The cat genes of Gram-negative and Gram-positive bacteria show little homology, and a variety of different enzymes have been described. The gene is most commonly found

on plasmids. Some times decreased outer membrane permeability or active efflux is observed in Gram-negative bacteria (Fluit *et al*., 2001).

1.17Antibiotic sensitivity

Antimicrobial resistance is now recognized as an increasingly global problem which was observed for the first time in *E.coli* 1940. The primary factor responsible for the development and spread of bacterial resistance is the injudicious use of antimicrobial agents (Atif *et al*., 2000).

Antibiotic usage is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant micro organism in both veterinary and human medicine. Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animal and human) or populations (Bogaard *et al*., 2001). Antibiotics are used in animals as in human for therapy and control of bacterial infections. In intensively reared food animals, antibiotics may be administered to whole flocks rather than individual animals. In addition antimicrobial agents may be continuously fed to food animals such as broiler as antimicrobial growth promoter. There for the antibiotic selection pressure for resistance in bacteria in poultry is high consequently their faecal flora contains a relatively high proportion of resistant bacteria (Bogaard *et al*., 2001).

Currently, there is increased public and scientific interest in the use of therapeutic and sub therapeutic antimicrobials in animals. This is due primarily to the possible emergence and dissemination of multiple-drug-resistant zoonotic bacterial pathogens. Antimicrobial drug-resistant bacterial pathogens in animals pose a risk not only to animal health but possibly to humans via transmission as food-borne pathogens (David *et al*., 2000).

Infections caused by antibiotic-resistant bacteria are a severe and costly animal health problem. These infections prolong illness and, if not treated in time with more costly, alternative antimicrobial agents, can lead to increased morbidity and mortality (David *et al*., 2000).

Multiple-drug resistance in enteric organisms like *E.coli* is known to be associated with integrons. Integrons generally contain an integrase gene (intI) and a cassette interation site (attI), into which antibiotic resistance gene cassette has integrated (Lydia *et al*., 1999).

Antibiotic resistant patterns (ABR) and transferability of the ABR markers was investigated in *E.coli* isolates obtained from drinking water and human urine samples. The ABR in *E.coli* isolates was determined against antibiotics commonly used in human and veterinary medicine. A high frequency of ABR to carbenicillin (56%), tetracycline (53%), and streptomycin (49%), and a low frequency of cefizoxime (5%), amikacine (8%), cefazidine (5%), chloramphenicol (9%), and kanamycin (18%) was found in the tested *E.coli* isolates (Walia *et al*., 2004).

Antibiotic resistance among avian bacterial isolates is common and is of great concern to the poultry industry. Approximately (36%) of avian pathogenic *E.coli* isolates obtained from diseased poultry exhibited multiple-antibiotic resistance to tetracycline, oxytetracycline, streptomycin, sulfonamides and gentamycin (Lydia *et al*., 1999).

Antibiotic have been used in the prevention and treatment of colibacillosis, particularly oxytetracycline, chlortetracycline, streptomycin and chloramphenicol, the greatest value has been derived when these substances are given orally for several days beginning with 24 hours of birth. It has been the practice on some farms to feed antibiotics as a prophylactic

measure but this has resulted in the development of resistant strains of *E.coli* (Buxton and Frazer, 1977).

The use of antibiotics incase of colibacillosis in poultry may be of limited value for financial reason and also because of the increasing problem of the development of drug resistant strains of *E.coli* (Buxton and Frazer, 1977).

Chulasiri and suthiekul (1989) illustrated that faecal *E.coli* isolates from healthy farm chickens, from chickens farm with avian influenza and and from chickens with diarrhea were more resistant to antimicrobial agents than those isolated from healthy domestic chickes. Transfer of drug resistance was ready achieved from strains isolated from both healthy and sick farm chickens, and from diarrheatic chickens. It was more difficult to demonstrate in strains from domestic chicken.

Frganis *et al*., (1989) reported that most isolates of *E.coli* obtained from postmortem materials of hens with septicemia were resistant to chloramphenicol, tetracycline, streptomycin, ampicillin, and erythromycin and trimethoprim suliamethoxadole but were highly sensitive to gentamycin and nalidixic acid.

The prophylactic use of sulphonamide to prevent coccidiosis (Smith.,*et al* 1973) may be responsible for the high proportion of *E.coli* in poultry, resistant to this chemotherapeutic agent.

Timms *et al*. (1989) evaluated the efficacy of chlortetracycline for the control of chronic respiratory disease caused by *E.coli* and *Mycoplasma gallisepticum* and found that medicated food and water reduced the severity of air saculitis and other postmortem lesion and lowered mortality. At slaughter, resistant strains from the gut readily soil poultry carcasses and as a

result poultry meats are often contaminated with multi resistant *E.coli* like wise eggs become contaminated during lying.

Resistant faecal *E.coli* from poultry can infect humans both directly and via food. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora (Bogaard et al., 2001).

The susceptibility of different *E.coli* isolates to different antibiotics at the specified concentration was studied (Kamal, thesis). All of the isolates were insensitive to penicillin, cloxacillin and erythromycin. Susceptibility of the different isolates varied from completely insensitive (resistant) to highly sensitive. Ninety four percent of the isolates tested were sensitive to chloramphenicol, 82% to ampicillin, 76% to streptomycine and 64% to tetracycline and co-trimoxazole. Streptomycin was the least active antibiotics against the isolates at the specified concentration. Susceptibility to tetracycline and co-trimoxazole was more or less the same. Resistance to both drugs was considerably high (more than35%). Also, pattern of susceptibility to ampicillin and chloramphenicol was comparable. Gentamicin was the only antibiotic to which all isolates were invariably sensitive.

The *invitro* activities of anti-microbial agents were determined against strains of *E.coli* isolated from lambs and kids affected by neonatal diarrhea. The over all percentage of resistant strains to streptomycine, sulphadimethoxine and tetracycline was very high (above 70%). A high level of resistant (from 30% to 50%) to ampicillin, kanamycin, neomycin and chloramphenicol was also detected. The *E.coli* strains were highly susceptible to cephalosporins, polymyxin and quinolones. Most of the strains showed multi resistance, 77.2% of isolates were resistant to at least

two antibiotics.55.4% were resistant to at least four antibiotics and 33.7 were resistant to at least six antibiotics (Cid *et al*., 1969).

Drug resistances of E.coli isolated from the intestinal tract of animals reflect their exposure to antibacterial drugs those select resistant clones. There is marked variation in resistance, which can be related to the antibacterial drugs used in animals in an area. Initial choice of an antibacterial must therefore be based on knowledge of usual patterns of resistance of *E.coli* isolates from a particular animal host in the area, and it is important to conduct antimicrobial susceptible to fluorquinolone, third generation cephalosporins, carbdox, gentamicin, trimethoprim, sulphaonamide and nitrofurans, isolates are likely to be resistant to streptomycin, sulphonamides and tetracyclines and amodrate percentage of strains are likely to be susceptible to chloramphenicol, ampicillin, neomycin and kanamycin (Gyles *et al*., 1993). *E.coli* that have multiple drug resistance patterns and are part of the normal flora may invade to produce septicaemia in calves and foals with failure passive transfer (radostitis et al., 1994).

Antibiotics should be given parent rally in case of acute mastitis caused by *E.coli*. Oxytetracycline, chlortetracycline and streptomycin given intramuscularly have been effective in the treatment of this condition.

Chauhan and Kaushik (1991) reported that the majority of isolates from enterotoxigenic *E.coli* from camel with diarrhea were susceptible to gentamycin, nitrofuranation, trimethoprin plus sulfonamide, neomycin, kanamycin and chloramphenicol.

Shears *et al*. (1987) investigates antibiotic resistance in gut commensals obtained from children presenting with diarrhea in Khartoum and Juba, *Escherichia coli* isolates were obtained from the faeces of children. Sensitivity to six antibiotics generally available in Sudan was

determined for all isolates using a disc diffusion method. The children isolates (74%) had resistant to at least four of the antibiotics and (20%) isolates had resistant to all six antibiotics. A greater number of resistant isolates was obtained from inpatients than out patients but were significantly different for only two antibiotics. There was no significant difference in the prevalence of resistant isolates obtained from children below 1 year of age. The results suggest that multiply resistant *E.coli* may be a quired from the environment, and may play a role in the epidemiology of multiply resistant enteric pathogens.

Newman and Seidu (2002) examined strains of *E.coli* investigate the frequency of resistance to eleven different antimicrobial agents, 68% of these isolates were resistant to tetracycline, and 57% were resistant to ampicillin and cotrimoxazole. One strain of *E.coli* was resistant to eight antimicrobials. Thirty percent of the *E.coli* was resistant to gentamicin.

Macias (2002) studied the rate of antibiotic resistance of fecal *E.coli* from healthy children. He found the heist rate of resistance was that to tetracycline, Ampicilline and trimethoprim. The resistance to ciprofloxacin, amikacin, gentamicin and ceftriaxone was less than 5%. Resistance to five or more antimicrobials was detected in 20.39% isolates.

E.coli strains isolated from patients with urinary tract infections were investigated for their antibiotic susceptibility. The strains were found to be highly susceptibility to gentamicin and bactrim (R) (Soyletir and Gumalp, 1985).

Antimicrobial sensitivity tests were performed on bacterial isolates from Sudanese patients with diarrhea or urinary tract infections. Entero-pathogenic *E.coli* showed high resistance rates against the commonly used antimicrobial agents: ampicillin, amoxicilline, chloramphenicol, tetracycline,

cotrimoxazole, nalidixic acid, sulfonamide and neomycin, and were complately sensitive to ciprofloxacin. Resistance to ampicillin, amoxicillin, tetracycline, cotrimoxazole and sulfonamide was the most frequent pattern. E.coli showed high rates of resistance to ampicillin, amoxicillin, cotrimoxazole, tetracycline, sulfonamide, trimethoprim, streptomycin, and carbenicillin (Ahmed *et al*., 2000).

CHAPTER TWO

2 MATERIAL AND METHODS

2.1 Sterilization

2.1.1 Flaming

It was used to sterilize glass slides, cover slips, needles and scalpels.

2.1.2 Red heat

It was used to sterilize loop wires, points and searing spatulas by holding them over Bunsen burner flame until became red-hot.

2.1.3 Hot air oven

It was used to sterilize glassware such as test tubes, graduated pipettes, flasks, forceps and cotton swabs. The holding period was one hour and oven temperature was 180ºC.

2.1.4 Steaming at 100ºC

Repeated steaming (Tyndallization) was used for sterilization of sugars and media that could not be autoclaved without deteriment effect to their constituents. It was carried out as described by Cruckshank *et al*. (1975).

2.1.5 Moist heat (Autoclave)

Autoclaving at 121ºC (151b/inch²) for 15 minutes was used for sterilization of media and plastic wares. Autoclaving at 115ºC (101b/inch²) for 10 minutes was used for sterilization of some media.

2.2 Reagents and indicators

2.2.1 Reagents:

2.2.1.1 Alpha-naphthol solution:

Alpha-naphthol is product of British Drug House, London (BDH). This reagent was prepared as 5% aqueous solution for Voges-Proskauer (VP) test.

2.2.1.2 Potassium hydroxide:

It was used for Voges-Proskauer test and was prepared according to Barrow and Feltham (1993) as 4% aqueous solution.

2.2.1.3 Hydrogen peroxide:

This reagent was obtained from Agropharm Limited, Buckingham. It was prepared as 3% aqueous solution and it was used for catalase test.

2.2.1.4 Methyl red reagent:

It was prepared by dissolving methyl red (0.04g) in ethanol (40ml). The volume was made to 100ml with distilled water. It was used for methyl red test.

2.2.1.5 Tetramethyl-p-phenylenediamine dihyadrochloride

This was prepared in a concentration of 30% aqueous solution and was used for oxidase test.

2.2.1.6 Kovac's reagent

This reagent composed of 5g para-dimethylaminobenzaldehyde, 75ml amyl alcohol and 25ml concentrated hydrochloric acid. It was prepared as described by Barrow and Feltham (1993) by dissolving the aldehyde in the alcohol by heating in water bath. It was then cooled and the acid was added. The reagent was stored at 4ºC for later use in indole test.

2.2.2 Indicators

2.2.2.1 Andrade's indicator

It composed of acidic fuchsin 5g, distilled water 1L and N-NaOH 150ml. The acid fuchsin was dissolved in distilled water, then the alkali solution was added, mixed and was allowed to stand at room temperature for 24h with frequent shaking until the color changed from red to brown.

2.2.2.2 Bromothymol blue

It was obtained from BDH. The solution was prepared by dissolving 0.2g of the bromothymol blue powder in 100ml distilled water.

2.2.2.3 Phenol red

It was obtained from Hopkins and William Ltd, London. It was prepared as 0.2% in distilled water.

2.2.3 Preparation of media

2.2.3.1 Nutrient broth

Thirteen grams of nutrient broth (Oxoid) were added to one liter of distilled water, mixed well and distributed in 3ml amount into clean test tubes, then sterilized by autoclaving at 121ºC for 15 minutes.

2.2.3.2 Peptone water

Fifty grams of peptone water powder (Oxoid) were added to one liter of distilled water, mixed well, distributed in 3ml amount into clean test tube and sterilized by autoclaving at 121ºC for 15 minutes.

2.2.3.3 Peptone water sugars

This medium was prepared according to Barrow and Feltham (1993). Nine hundred ml of peptone water were prepared and the pH was adjusted to 7.1- 7.3. Ten ml of Andrade's indicator were added.

Sugar solution was prepared by dissolving 10 grams of the appropriate sugar in 90 ml of peptone water. This sugar solution was added to the peptone water, distributed in 5 ml volume into sterile test tubes with inverted Durham's tubes and sterilized by autoclaving at 115ºC for 10 minutes.

2.2.3.4 Glucose phosphate medium (MR-VP test medium)

This medium was prepared according to Barrow and Feltham (1993). Peptone 5g and 5g of phosphate buffer (K_2HPO_4) were added to one liter of distilled water, dissolved by steaming, filtered and pH was adjusted to 7.5. Then five grams of glucose were added, mixed well, distributed into clean test tubes and sterilized by autoclaving at 115ºC for 15 minutes.

2.2.3.5 Nutrient agar

To one liter of nutrient broth (Oxiod) 15g of agar were added, dissolved by boiling, sterilized by autoclaving at 121ºC for 15 minutes. Then cooled to about 50ºC and distributed in 15ml amount per plate. The poured plates were left to solidify at room temperature on leveled surface.

2.2.3.6 Diagnostic sensitivity test agar

This medium was supplied by Oxiod. It consists of protease peptone, veal infusion solids, dextrose, sodium chloride, disodium phosphate, sodium acetate, adenine sulphate, guanine hydrochloride, uracil, xanthine, and ion agar No.2.

Forty grams of the medium were suspended in one liter of distilled water, and then brought to boil to dissolve completely, sterilized by autoclaving at 121ºC for 15 minutes, then dispended into sterile Petri dishes in portions of 15ml each. The poured plates were left to solidify at room temperature on leveled surface.

2.2.3.7 MacConkey's agar

Fifty two grams of MacConkey's agar (Oxoid) were suspended in one liter of distilled water, brought to boil to dissolve the ingredients completely, then sterilized by autoclaving at 121ºC for 15 minutes and poured into sterile Petri dishes in 15ml amount. The poured plates were left to solidify at room temperature on flat surface.

2.2.3.8 Motility medium-Cragie tube medium

Thirteen grams of dehydrated nutrient broth (Oxoid) were added to 5 grams of Oxoid agar No-1 and dissolved in one liter of distilled water. The pH was adjusted to 7.4. This medium was dispensed in volumes of 5ml into 20ml test tubes containing the appropriate Cragie tubes, then the medium in the test tubes were sterilized by autoclaving at 121ºC for 15 minutes.

2.2.3.9 Hugh and Leifson's (O/F) medium

This medium was prepared as described by Barrow and Feltham (1993). Two grams of peptone powder, five grams of sodium chloride, 0.3g of potassium hypophosphate and three grams of agar were added to one liter of distilled water, then heated in water bath at 55ºC to dissolve the solids. The pH was adjusted to 7.1 and filtered. Then the indicator bromothymol blue (0.2% aqueous solutions) was added and the mixture was sterilized at 115ºC for 10 minuets. Filtered sterile glucose solution was added aseptically to give final concentration of 1%. Then the medium was mixed and distributed aseptically in 10ml amount into sterile test tubes of not more than 16mm diameter.

2.2.3.10 Urea agar medium

This medium was supplied by Oxoid. It consist of peptone (1g), dextrose (1g), Na_2HPO_4 (1.2g), KH_2PO_4 (0.8), sodium chloride (5g), Agar (15g) and phenol red (0.012g). Dehydrated medium (2.4g) were suspended

in 95 ml of distilled water and brought to boil to dissolve completely. The pH was approximately adjusted to 6.8 and then it was sterilized by autoclaving at 115ºC for 20 minutes. Then cooled to 50ºC and aseptically 5ml of sterile 40% urea solution was added and mixed well. The medium was then distributed in 10 ml amounts into sterile McCartney bottles and allowed to set in a slope position.

2.2.3.11 Simmon's citrate medium

The dehydrated medium of (Difco) consist of ammonium dihydogen phosphate (1g), magnesium sulphate (0.2g), K_2HPO_4 (1g), sodium citrate (2g), sodium chloride (5g), Bacto-agar (15g) and Bacto-bromothymol blue(0.08g).

According to the manufacture 24g of the medium were dissolved in one liter of distilled water and pH was adjusted to 6.8. Then sterilized by autoclaving at 115ºC for 20 minutes, distributed into MacCarteny bottles and allowed to solidify in a slope position.

2.2.4 Collection of samples

A total of one hundred and ninety samples were collected. One hundred samples were collected from infected poultry, twenty from calf feces showed diarrhea, fifteen from mastitic cow's milk, fifteen from women urine with urinary tract infection and ten from bovine abscesses. Thirty swab samples were collected from child diarrhea patients at Omdurman Pediatric hospital.

2.2.5 Transportation of samples

Samples were collected in sterile MacCarteny bottles and labeled and kept on ice. All samples were cultured 2-3 hours after collection or kept frozen at -20ºC.

2.2.6 Culture of specimens

The collected samples were inoculated onto MacConkey agar. The inoculated plates were then incubated for 24 hours at 37ºC.

After the incubation period the characteristic colonies were observed and smears were made from lactose fermenting, red or pink colonies. Then dried in the air, fixed by heating, stained by Grams method and examined under light microscope for cell morphology and staining reaction.

2.2.7 Purification

Isolates were purified by several subculturing from single well-separated colony on nutrient agar plates. The purity was checked by examining Gram stained smear. The pure culture was then used for studying cultural and biochemical characteristics and antibiotics' sensitivity.

2.2.8 Microscopic examination

Smears were made from colonies on primary culture and from purified colonies, fixed by heating and stained by Gram method (Barrow and Feltham, 1993), then examined microscopically by oil immersion. The smear was examined for cell morphology and staining reaction.

2.2.9 Identification of bacteria

The purified isolates were identified according to the criteria described by Barrow and Feltham (1993). This included staining reaction, organism morphology, growth condition, and the colonies characteristics on different media, motility and biochemical characteristics.

2.2.10 Biochemical methods

2.2.10.1 Catalase test

The test was carried out as described by Barrow and Feltham (1993). A drop of 3% aqueous solution of hydrogen peroxide was placed on a clean

slide. Small amount of the tested organism colony on nutrient agar was picked by glass rod, added to the drop and mixed. Production of air bubble indicated positive result.

2.2.10.2 Oxidase test

The method of Barrow and Feltham (1993) was used. Strip of filter paper was soaked in 1% solution of tetra methyl-p-phenylene diamine dihydrochloride and dried in hot air oven and then placed on clean glass slide by sterile forceps. A fresh young tested culture on nutrient agar was picked off with sterile glass rod and rubbed on the filter paper strip. If a purple color developed within 5-10 seconds, the reaction was considered positive.

2.2.10.3 Oxidation- fermentation test

The test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated with straight wire into duplication of test tubes of Hugh and Leifeson's medium. To one of the test tube a layer of melted soft paraffin oil was added to the medium to seal it from air. The inoculated tubes were incubated at 37ºC and examined daily for fourteen days. Yellow color in open tube only indicated oxidation of glucose; yellow color in both tubes showed fermentation reaction and blue or green color in open tube indicated production of alkali.

2.2.10.4 Sugar fermentation test

The test was carried out as described by Barrow and Feltham (1993). The peptone water sugar was inoculated with organism under the test, incubated at 37ºC and then examined daily for several days. Acid production was indicated by appearance of redish color, while gas production was indicated by presence of empty space in the inverted Durham's tubes.

2.2.10.5 Citrate utilization

To test the ability of the test organism to utilize citrate as a sole source of carbon, heavy inoculum was cultured on the surface of a slope of Simmon's citrate medium and incubated at 37ºC and examined daily. A positive test was indicated by change of color from green to blue. A negative test was not considered before end of 14 days.

2.2.10.6 Urease activity

Urease activity is shown by alkali production (ammonia) from urea splitting by the test organism. Heavy inoculum was cultured on the surface of urea agar slope and incubated at 37ºC and examined daily. A positive test was indicated by pink or red color. A negative result was not considered before end of 7 days.

2.2.10.7 Indole production test

Indole production test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated into peptone water and incubated at 37ºC for 48 h. One milliliter of the Kovacs reagent was run down along side of the test tube. Appearance of pink color in the reagent layer within a minute indicated positive reaction.

2.2.10.8 Methyl red test

Methyl red test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated into glucose phosphate medium (MR.VP medium), then incubated at 37ºC for 48 h. Two drops of methyl red reagent were added, shaken well and examined. Appearance of red color indicated positive reaction.

2.2.10.9 Voges- Proskauer test

The test was performed as described by Barrow and Feltham (1993). The test culture was inoculated into glucose phosphate medium (MR.VP

medium) and incubated at 37ºC for 48 hours. Then 0.6 ml of 5% α- naphthol followed by a 0.2 ml of 40% potassium hydroxide aqueous solution were added to one ml of the culture, shaked well and examined after 15 minutes. A positive reaction was indicated by appearance of bright pink colour.

2.2.10.10 Motility test

Craigi tube in semi- solid nutrient agar, prepared as described before (2.2.3.8). A small piece of the colony of the bacterium under test, was picked by the end of the straight wire and stabbed in the center of semi- sold agar in the Craigi tube and then incubated at 37ºC overnight. The organism was considered motile if there was turbidity in the medium in and outside the Craigi tube.

2.2.10.11 Antibiotic sensitivity

Sensitivity of isolates to a number of antibiotics was determined by disc diffusion technique (Cruckshank *et al*., 1975). The isolate was grown on peptone water and incubated at 37ºC for two hours. One to two ml of the culture was poured on a Petri dish containing diagnostic sensitivity test agar medium (DST) and dried in oven at 40ºC for 20 minutes before the inoculation. The inoculum was evenly distributed by rotation. Excess fluid was withdrawn using sterile Pasteur pipette and plate was let to dry at room temperature for 15 minutes. Commercial discs obtained from Axiom Laboratories (India) were placed on the surface of the medium by sterile forceps, pressed gently to ensure full contact with the surface of the culture medium. The plates were then incubated at 37ºC for 24 hours and up to 48 h. After incubation, the plates were examined for growth inhibition around disc and the diameter of zone of growth inhibition was measured in millimeters. The antibiotics examined in this study were: amoxicillin 10µg , amikacin 30µg, ceftizoxime 30µg, chloramphenicol 30µg, cephalexin 30µg,

tetracycline 30µg, ciprofloxacine 5µg, nitrofuranation 300µg, nalidixic acid 30µg, gentamicin 10µg, norfloxacin 10µg, ofloxacin 5µg, ampicillin 20µg, co- trimoxazole 25µg and pefoxacin 10µg (Table 1).

2.2.10.12 Isolation of plasmid DNA

To confirm the presence of extrachromosomal DNA in *E.coli* strains, it was necessary to isolate plasmid DNA and to detect it using conventional Agarose electrophoresis. A modified version from the methods described by Kado (1981) and Oslen (1990) was used to isolate plasmid DNA. Overnight culture was harvested by centrifugation at 2500 g/10 min and resuspended in 40 µl 50 mM tris EDTA, pH 8.0. The cells were lysed at 56 ºC/1 h in 400 µl 3% SDS, 50 mM tris, pH 12.5. After addition of 300 µl 1.5 M K acetate, pH 5.2, the suspension was left for 20 min on ice. The supernatant fluid was cleared from the precipitate by centrifugation at 2400 g/10 min. The supernatant was treated once with an equal volume of chloroform: phenol (1:1, v/v). The phases were separated by centrifugation for 15 min at 2400 g/4 ºC and the upper phase was transferred to tube. 100 µl of 3 M sodium acetate and 800 µl of 96% ethanol were added and the mixture was left at 4 ºC for 30 min. Plasmid DNA was collected by centrifugation at 6720 g/15 min at 2 ºC and resuspended in 100 µl buffer or autoclaved water.

Five strains of *E.coli*, 67b (sensitive), 131 (resistant to two antibiotics), 71 (resistant to four antibiotics), 10 (resistant to six antibiotics) and 67a (resistant to eight antibiotics) were examined.

2.2.10.13 Detection of plasmid DNA

Twenty µl of aqueous phase containing native plasmid was mixed with 5 µl of 0.025 % bromophenol blue (MERCK) and xylene cyanole FF (SIGMA) in 50 % glycerol in TAE buffer (40 mM Tris, 5mM sodium

acetate, 1mM EDTA, pH 8) and separated for 2 h on 0.7 % horizontal Agarose gel at a current of 100 V in 1x TAE buffer.

Ladder (100 bp) was used as molecular weight marker. Gels were stained with ethidium bromide (0.25 µg/ml) photographed under UV light and printed.

CHAPTER THREE

3 RESULTS

3.1 Survey

A total of one hundred and ninety samples were collected. One hundred samples were collected from infected poultry tissues, twenty from feces of calves showing diarrhea, fifteen from milk of mastitic cow's, fifteen from urine of women with urinary tract infection, ten from bovine abscess and thirty swab samples were collected from cases of child diarrhea at Omdurman pediatric hospital.

Collected samples were cultured onto MacConkey agar and incubated aerobically at 37ºC.

E.coli was isolated from eighty one poultry samples, twelve calf fecal samples, nine mastitic cow's milk, eight women urine, two bovine abscess and twenty child diarrhea samples (Table 1).

3.2 Growth on solid media

A MacConkey's agar

On MacConkey's agar medium large, profuse, smooth pink colonies, 2-3 mm in diameter were seen (Figure 1).

B Nutrient agar

On nutrient agar colonies were small and more mucoid than on MacConkey's agar (Figure 2).

3.3 Microscopic and cultural characteristics of the isolates

Microscopic examination of isolates revealed Gram-negative short rods, occurring singly or in pairs.

3.4 Growth in liquid media

A heavy turbidity was produced by all the isolates in peptone water following overnight incubation. There was a heavy deposit which was easily dispersed by shaking. Incubation of cultures for six hours resulted in slightly turbid growth without deposit.

3.5 Biochemical characteristics of the isolates

3.5.1 Oxidase test

All the isolates were oxidase negative.

3.5.2 Catalase test

All isolates were catalase positive, producing O_2 gas that appears in the form of bubbles.

3.5.3 Sugar fermentation test

On sorbitol all strains of *E.coli* fermented sorbitol with production of acid and gas, and fermented glucose with production of acid and gas. Acid was indicated by change in the colour of the medium to pink. Gas production was indicated by appearance of air bubbles at the top of Durham's tube.

3.5.4 Oxidation fermentation test

All isolates of *E.coli* were oxidative and fermentative.

3.5.5 Indole test

All strains of *E.coli* examined were able to produce indole when inoculated into peptone water.

3.5.6 Voges-Proskauer test

All strains of *E.coli* were unable to produce acetyl methyl carbinol in phosphate glucose medium and hence the colour of the medium was not

changed to red when alpha- naphthol and potassium hydroxide were added to the tested culture.

3.5.7 Methyl red test

All strains of *E.coli* were methyl red positive.

3.5.8 Citrate utilization test

All strains were unable to utilize citrate when inoculated into Simmon's citrate medium.

3.6 Antibiotic sensitivity:

The antibiotic sensitivity of *E.coli* isolates of this study was examined against 15 antibacterial agents by using the disc diffusion technique (Figure 3).

Infected poultry isolates were completely sensitive to amikacin (100%) and ceftriaxom (100%). While child diarrhea isolates were completely sensitive to cephalexin (100%), nitrofuranation (100%), nalidixic acid (100%) and ofloxacin (100%). Also women urine isolates were completely sensitive to amikacin (100%), ceftizoxim (100%), chlormaphenicol (100%), nitrofuranation (100%) and pefoxacin (100%).

Mastitic cow's milk isolates were completely sensitive (100%) to amikacin, ceftizoxim, chlormaphenicol, cephalexin, ciprofloxacin, nitrofuranation, nalidixic acid, gentamicin, norfloxacin, ofloxacin, ampicillin pefolxacin and co- trimoxazole, except amoxicillin (67%) and tetracycline (67%) showed resistance.

Child diarrhea isolates showed some resistance to amoxicillin (60%), tetracycline (70%), norfloxacin (55%), ampicillin (55%) and co- trimoxazol (55%). While nalidixic acid showed complete effectiveness (100%) against child diarrhea isolates.

Women urine isolates showed high resistance to tetracycline (75%), and intermediate susceptibility to amoxicillin (50%), ampicillin (50%) and co- trimoxazol (50%) and showed high susceptibility (75%) to gentamicin.

Calf feaces isolates were completely sensitive (100%) to amikacin, ceftizoxim, chlormaphenicol, cephalexin, nitrofuranation, gentamicin, ofloxacin, ampicillin, pefolxacin and co- trimoxazole.

Also calf feaces isolates showed high sensitivity to ciprofloxacin (92%), norfloxacin (92%), nalidixic acid (75%) and showed intermediate sensitivity to tetracycline (58%).

Bovine abscess isolates were completely sensitive (100%) to most antibiotics however, bovine sove isolates were completely resistant (100%) to amoxicillin, tetracycline, nalidixic acid, ofloxacin. While ciprofloxacin and norfoxacin showed intermediate effectiveness (50%).

The multiple drug- resistance observed in this study varied from two drugs multiple resistances (tetracycline and nalidixic acid) to nine drugs multiple resistances (chloramphenicol, cephalexin, tetracycline, ciprofloxacin, nalidixic acid, norfloxacin, ofloxacin, ampicillin and pefolxacin).

E.coli strains showed the highest multiple drugs resistance were isolated from infected poultry tissues (up to 9 antimicrobial drugs) women urine (up to 7 antimicrobial drugs) and bovine abscess (up to 6 antimicrobial drugs).

Most of the strains examined showed multi- resistance; as 75.7% of isolates were resistant to at least two antibiotics, 59.8% were resistant to at least three antibiotics, 43.1% were resistant to at least four antibiotics, 25.7% were resistant to at least five antibiotics, 18.9% were resistant to at least six

antibiotics, 9% to at least 7 antibiotics, 3% to at least 8 antibiotics and 1.5% to at least 9 antibiotics.

The sensitivity of tested isolates to the different antibacterial agents was variable (Tables 2, 3, 4, 5, 6, 7, 8). Some isolates were sensitive to all antibacterial agents 10 (7.5%), while others were resistant to one antibacterial agent 22 (16.6%), two antibacterial agents 21 (15.9%), three antibacterial agents 22 (16.6%), four antibacterial agent 23 (17.4%), five antibacterial agents 9 (6.85), six antibacterial agents 13 (9.8%), seven antibacterial agents 8 (6%), eight antibacterial agents 2 (1.5%) and nine antibacterial agents 2 (1.5%) (Table 9).

3.7 Detection of plasmid DNA

In this study, five *E.coli* isolates were examined for plasmid DNA. The isolates did not show clear bands of plasmid (Figure 4).

Table 1: Isolation of *E.coli* from different sources

Source of samples	**Number of samples examined**	**Number of isolates recovered**	**Isolation rate**
Infected poultry tissues	100	81	81 %
Calf fecal sample	20	12	60 %
Mastitic cow's milk	15	9	60 %
Women urine	15	8	53.3 %
Bovine abscess	10	2	20 %
Child diarrhea	30	20	66.7%
Total	190	132	69.47%

Table 2: Antibiotic sensitivity of *E.coli* isolated from different sources

Antibiotic	Number of bacteria examined	No. of isolates Sensitive (percentage)	No. of isolates Resistant (percentage)
Amoxicillin AM- 10 mcg	132	80 (61%)	52 (39%)
Amikacin AK- 30 mcg	132	131 (99%)	1 (1%)
Ceftizoxim CI- 30 mcg	132	131 (99%)	1 (1%)
Chloramphenicol CH- 30 mcg	132	96 (73%)	36 (27%)
Cephalexin PR- 30 mcg	132	125 (95%)	7 (5%)
Tetracycline TE- 30 mcg	132	29 (22%)	103 (78%)
Ciprofloxacin CP- 5 mcg	132	104 (78%)	28 (22%)
Nitrofurantion FD- 300 mcg	132	126 (95%)	6 (5%)
Nalidixic acid NA- 30 mcg	132	55 (42%)	77 (58%)
Gentamicin GM- 10 mcg	132	117 (89%)	15 (11%)
Norfloxacin NX- 10 mcg	132	104 (78%)	28 (22%)
Ofloxacin OF- 5 mcg	132	103 (78%)	29 (22%)
Ampicillin AS- 20 mcg	132	112 (85%)	20 (15%)
Co- Trimoxazole BA- 25 mcg	132	79 (60%)	53 (40%)
Pefloxacin PF- 10 mcg	132	96 (73%)	36 (27%)

Table 3: Antibiotics sensitivity of *E.coli* isolated from infected poultry tissues

Antibiotic	No. of isolates examined	No. of isolates sensitive (percent)	No. of isolates resistant (percent)
Amoxicillin AM- 10 mcg	81	64 (79%)	17 (30%)
Amikacin AK- 30 mcg	81	81 (100%)	0 (0%)
Ceftizoxim CI- 30 mcg	81	81 (100%)	0 (0%)
Chloramphenicol CH- 30 mcg	81	47 (58%)	34 (42%)
Cephalexin PR- 30 mcg	81	75 (93%)	6 (7%)
Tetracycline TE- 30 mcg	81	11 (14%)	70 (86%)
Ciprofloxacin CP- 5 mcg	81	62 (77%)	19 (23%)
Nitrofurantion FD- 300 mcg	81	75 (93%)	6 (7%)
Nalidixic acid NA- 30 mcg	81	12 (15%)	69 (85%)
Gentamicin GM- 10 mcg	81	69 (85%)	12 (15%)
Norfloxacin NX- 10 mcg	81	67 (83%)	14 (17%)
Ofloxacin OF- 5 mcg	81	57 (70%)	24 (30%)
Ampicillin AS- 20 mcg	81	74 (91%)	7 (9%)
Co- Trimoxazole BA- 25 mcg	81	41 (51%)	40 (49%)
Pefloxacin PF- 10 mcg	81	45 (56%)	36 (44%)

Table 4: Antibiotics sensitivity of *E.coli* isolated from calf fecal samples

Antibiotic	No. of isolates examined	No. of isolates sensitive (percent)	No. of isolates resistant (percent)
Amoxicillin AM- 10 mcg	12	1 (8%)	11 (92%)
Amikacin AK- 30 mcg	12	12 (100%)	0 (0%)
Ceftizoxim CI- 30 mcg	12	12 (100%)	0 (0%)
Chloramphenicol CH- 30 mcg	12	12 (100%)	0 (0%)
Cephalexin PR- 30 mcg	12	12 (100%)	0 (0%)
Tetracycline TE- 30 mcg	12	7 (58%)	5 (42%)
Ciprofloxacin CP- 5 mcg	12	11 (92%)	1 (8%)
Nitrofurantion FD- 300 mcg	12	12 (100%)	0 (0%)
Nalidixic acid NA- 30 mcg	12	9 (75%)	3 (25%)
Gentamicin GM- 10 mcg	12	12 (100%)	0 (0%)
Norfloxacin NX- 10 mcg	12	11 (92%)	1 (8%)
Ofloxacin OF- 5 mcg	12	12 (100%)	0 (0%)
Ampicillin AS- 20 mcg	12	12 (100%)	0 (0%)
Co- Trimoxazole BA- 25 mcg	12	12 (100%)	0 (0%)
Pefloxacin PF- 10 mcg	12	12 (100%)	0 (0%)

Table 5: Antibiotics sensitivity of *E.coli* isolated from mastitic cow's milk

Antibiotic	No. of isolates examined	No. of isolates sensitive (percent)	No. of isolates resistant (percent)
Amoxicillin AM- 10 mcg	9	3 (33%)	6 (67%)
Amikacin AK- 30 mcg	9	9 (100%)	0 (0%)
Ceftizoxim CI- 30 mcg	9	9 (100%)	0 (0%)
Chloramphenicol CH- 30 mcg	9	9 (100%)	0 (0%)
Cephalexin PR- 30 mcg	9	9 (100%)	0 (0%)
Tetracycline TE- 30 mcg	9	3 (33%)	6 (67%)
Ciprofloxacin CP- 5 mcg	9	9 (100%)	0 (0%)
Nitrofurantion FD- 300 mcg	9	9 (100%)	0 (0%)
Nalidixic acid NA- 30 mcg	9	9 (100%)	0 (0%)
Gentamicin GM- 10 mcg	9	9 (100%)	0 (0%)
Norfloxacin NX- 10 mcg	9	9 (100%)	0 (0%)
Ofloxacin OF- 5 mcg	9	9 (100%)	0 (0%)
Ampicillin AS- 20 mcg	9	9 (100%)	0 (0%)
Co- Trimoxazole BA- 25 mcg	9	9 (100%)	0 (0%)
Pefloxacin PF- 10 mcg	9	9 (100%)	0 (0%)

Table 6: Antibiotics sensitivity of *E.coli* isolated from women urine

Antibiotic	No. of isolates examined	No. of isolates sensitive (percent)	No. of isolates resistant (percent)
Amoxicillin AM- 10 mcg	8	4 (50%)	4 (50%)
Amikacin AK- 30 mcg	8	8 (100%)	0 (0%)
Ceftizoxim CI- 30 mcg	8	8 (100%)	0 (0%)
Chloramphenicol CH- 30 mcg	8	8 (100%)	0 (0%)
Cephalexin PR- 30 mcg	8	7 (88%)	1 (12%)
Tetracycline TE- 30 mcg	8	2 (25%)	6 (75%)
Ciprofloxacin CP- 5 mcg	8	5 (63%)	3 (37%)
Nitrofurantion FD- 300 mcg	8	8 (100%)	0 (0%)
Nalidixic acid NA- 30 mcg	8	5 (63%)	3 (37%)
Gentamicin GM- 10 mcg	8	6 (75%)	2 (25%)
Norfloxacin NX- 10 mcg	8	5 (63%)	3 (37%)
Ofloxacin OF- 5 mcg	8	5 (63%)	3 (37%)
Ampicillin AS- 20 mcg	8	4 (50%)	4 (50%)
Co- Trimoxazole BA- 25 mcg	8	4 (50%)	4 (50%)
Pefloxacin PF- 10 mcg	8	8 (100%)	0 (0%)

Table 7: Antibiotics sensitivity of *E.coli* isolated from bovine abscess

Antibiotic	No. of isolates examined	No. of isolates sensitive (percent)	No. of isolates resistant (percent)
Amoxicillin AM- 10 mcg	2	0 (0%)	2 (100%)
Amikacin AK- 30 mcg	2	2 (100%)	0 (0%)
Ceftizoxim CI- 30 mcg	2	2 (100%)	0 (0%)
Chloramphenicol CH- 30 mcg	2	2 (100%)	0 (0%)
Cephalexin PR- 30 mcg	2	2 (100%)	0 (0%)
Tetracycline TE- 30 mcg	2	0 (0%)	2 (100%)
Ciprofloxacin CP- 5 mcg	2	1 (50%)	1 (50%)
Nitrofurantion FD- 300 mcg	2	2 (100%)	0 (0%)
Nalidixic acid NA- 30 mcg	2	0 (0%)	2 (100%)
Gentamicin GM- 10 mcg	2	2 (100%)	0 (0%)
Norfloxacin NX- 10 mcg	2	1 (50%)	1 (50%)
Ofloxacin OF- 5 mcg	2	0 (0%)	2 (100%)
Ampicillin AS- 20 mcg	2	2 (100%)	0 (0%)
Co- Trimoxazole BA- 25 mcg	2	2 (100%)	0 (0%)
Pefloxacin PF- 10 mcg	2	2 (100%)	0 (0%)

Table 8: Antibiotics sensitivity of *E.coli* isolated from child diarrhea

Antibiotic	No. of isolates examined	No. of isolates sensitive (percent)	No. of isolates resistant (percent)
Amoxicillin AM- 10 mcg	20	8 (40%)	12 (60%)
Amikacin AK- 30 mcg	20	19 (95%)	1 (5%)
Ceftizoxim CI- 30 mcg	20	19 (95%)	1 (5%)
Chloramphenicol CH- 30 mcg	20	18 (90%)	2 (10%)
Cephalexin PR- 30 mcg	20	20 (100%)	0 (0%)
Tetracycline TE- 30 mcg	20	6 (30%)	14 (70%)
Ciprofloxacin CP- 5 mcg	20	16 (80%)	4 (20%)
Nitrofurantion FD- 300 mcg	20	20 (100%)	0 (0%)
Nalidixic acid NA- 30 mcg	20	20 (100%)	0 (0%)
Gentamicin GM- 10 mcg	20	19 (95%)	1 (5%)
Norfloxacin NX- 10 mcg	20	11 (55%)	9 (45%)
Ofloxacin OF- 5 mcg	20	20 (100%)	0 (0%)
Ampicillin AS- 20 mcg	20	11 (55%)	9 (45%)
Co- Trimoxazole BA- 25 mcg	20	11 (55%)	9 (45%)
Pefloxacin PF- 10 mcg	20	20 (100%)	0 (0%)

Table 9: Multi- antibiotics resistance of *E.coli* isolated from different sources

No. of antibiotics resistant to	No. of isolates examined	No. of multi-resistance isolates (percentage)	Source of isolation
O	132	10 (7.5%)	A– B- C- D– F
1	132	22 (16.6%)	A– B- C- D– F
2	132	21 (15.9%)	A– B- C- F
3	132	22 (16.6%)	A– B- F
4	132	23 (17.4%)	A– D– E- F
5	132	9 (6.8%)	A– B- F
6	132	13 (9.8%)	A– D– E
7	132	8 (6%)	A– D
8	132	2 (1.5%)	A
9	132	2 (1.5%)	A
10	132	O (0%)	-
11	132	O (0%)	-
12	132	O (0%)	-

A: Infected poultry tissues
B: Calf fecal samples
C: Mastitic cow's milk
D: Women urine
E: Bovine abscess
F: Child diarrhea

Fig. 1: *E.coli* on MacConkey's agar

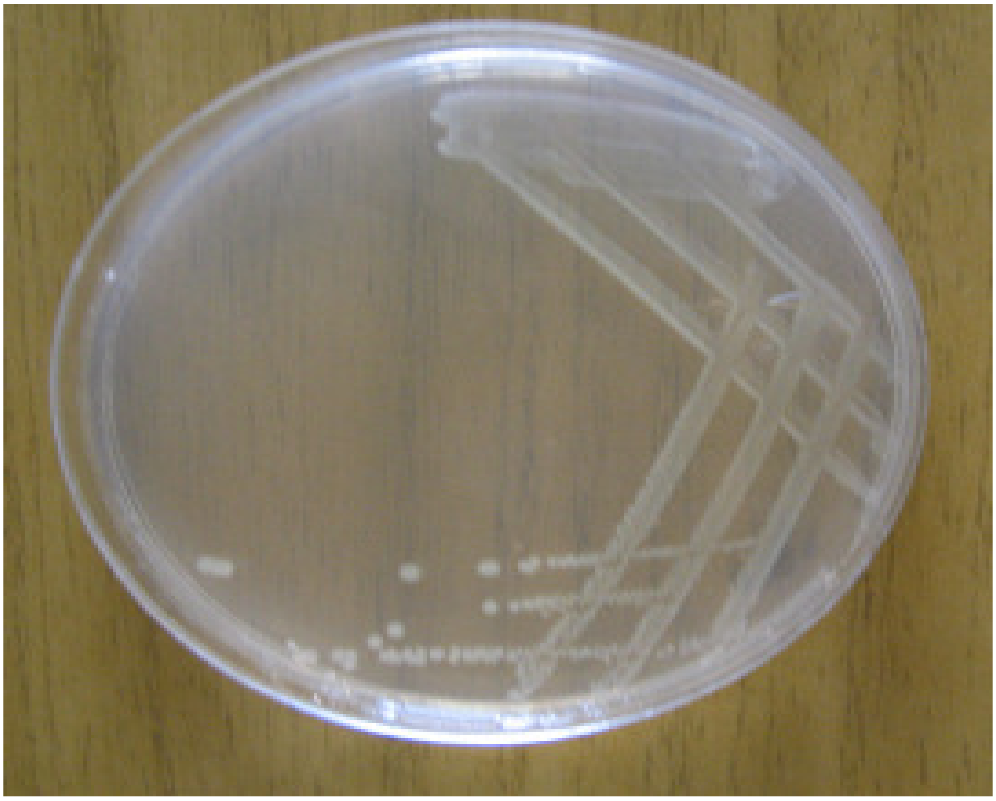

Figure 2: *E.coli* on Nutrient agar

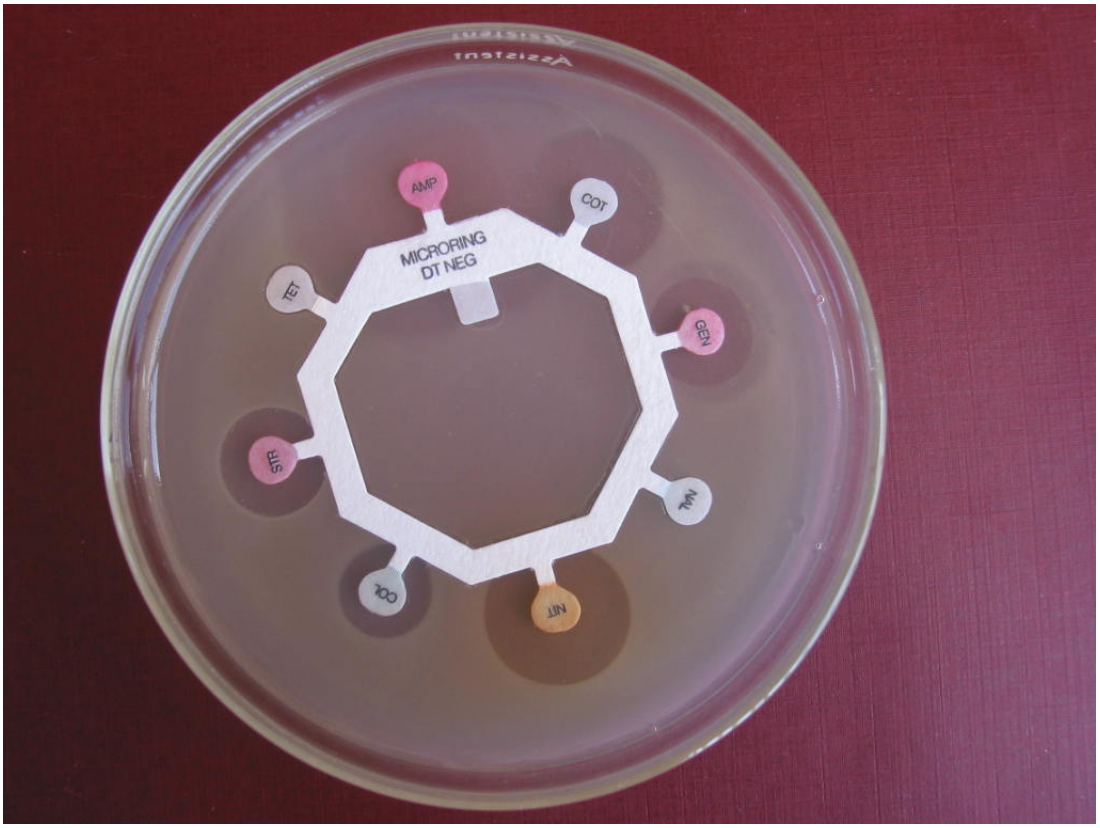

Fig.3: Antibiotics sensitivity of *E.coli*.

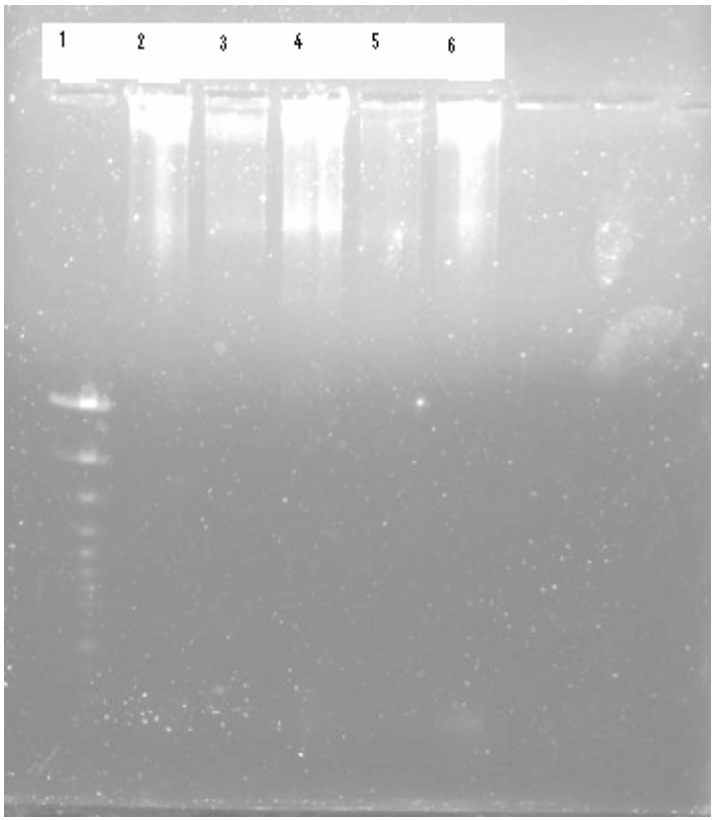

Fig 4: Agarose gel (0.7%) electrophoresis of plasmid DNA from *E.coli* isolates. lane 1 ladder plasmid (100 bp), lane 2 strain 67b (sensitive), lane 3 strain 131(2 drugs resistant), lane 4 strain 71(4 drugs resistant), lane 5 strain 10 (6 drugs resistant), lane 6 strain 67a (8 drugs resistant).

CHAPTER FOUR

DISCUSSION

This study was carried out to detect the drug- resistance in *E.coli* isolated in Khartoum State and also to compare the plasmids size of *E.coli* isolates having different multiple drug resistance.

Eighty-one isolates of *E.coli* were obtained from infected poultry tissues. This result is in agreement with Bass *et al*. (1999) who reported that *E.coli* adversely affects avian species through infection of the blood, respiratory tract, and soft tissues.

This study showed a high prevalence of antimicrobial drug resistance in *E.coli* isolated from poultry. Infected poultry isolates showed resistance to tetracycline (86%) and nalidixic acid (85%). This result confirms the finding of Bass (1999) who reported antibiotic resistance among avian bacterial isolates and most isolates were resistance to tetracycline. Also this finding agrees with El- Gasim (1999) who observed resistance to nalidixic acid among poultry isolates. This may indicate increasing in the use of nalidixic acid whether in treatment of colibacillosis or poultry formulas.

Eight isolates of *E.coli* were obtained from women with urinary tract infection. This finding agrees with El sheikh (2004) who reported the presence of *E.coli* in pregnant women with urinary tract infection.

Women urine isolates showed susceptibility to amikacin (100%), ciprofloxacin (63%), nitrofuranation (100%), gentamicin (75%) and norfoxacin (63%). This finding agrees with Jose *et al*. (2002) who reported that, *E.coli* isolated from urinary tract infections showed high susceptibility to amikacin (97%), gentamicin (94%), nitrofurantoin (89%), norfloxacin

(81%) and ciprofloxacin (78%). Also this finding agrees with Soyletir and Gumalp (1985) who found that *E.coli* strains isolated from patients with urinary tract infections were susceptible to gentamicin (75%). The isolates of this study showed medium susceptibility (50%) to ampicillin. This result is similar to that reported by Jose *et al*. (2002) who stated that, there was a low susceptibility pattern of *E. coli* isolated from urinary tract infections to ampicillin (41%).

Women urine isolates of this study showed resistance to tetracycline (75%). Which is contrast to the finding of El sheikh (2004) who stated that (50%) of the *E.coli* strains obtained from pregnant women with urinary tract infection were resistant to tetracycline. Most of the strains showed multi-resistance (87.8%) this finding agrees with those of Guyot *et al*., (1999) who stated that, thirty- two multi- resistant *E.coli* strains were isolated from the urine of thirteen patients with chronic urinary tract infection.

Twelve isolates of *E.coli* were obtained from calf feaces. This finding agrees with Ellaithi (2004) who reported the isolation of *E.coli* from 80% of faeces of diarrhoic calves in the Sudan.

Calf feaces *E.coli* isolates of this investigation were completely sensitive to gentamicin (100%). This finding agrees with Altom (2000) who reported that *E.coli* isolates from animals with diarrhea were sensitive to gentamicin. Also this finding is in agreement with Orden (2000) who found that, *E.coli* strains isolated from diary calves affected by neonatal diarrhea were very susceptible (89- 95%) to gentamicin.

Nine isolates of *E.coli* were obtained from mastitic cow's milk. This result is in agreement with that of Elgadasi (2003) who isolated *E.coli* from mastitic milk of cattle, sheep and goats collected 3 days after treatment, in Khartoum State.

Mastitic cow's milk isolates were completely sensitive to amoxicillin (100%), ceftizoxime (100%), chloramphenicol (100%), cephalexin (100%), ciprofloxacine (100%), nitrofuranation (100%), nalidixic acid (100%), gentamicin (100%), norfloxacin (100%), ofloxacin (100%), ampicillin (100%), co- trimoxazole (100%) and pefoxacin (100%). This finding agrees with Mohamed (2005) who stated that *E.coli* isolated from mastitic cow's milk showed complete sensitivity to gentamicin (100%), nalidixic acid (100%), nitrofuranation (100%). However, Elgadasi (2003) isolates were less sensitive than isolates of this study to chloramphenicol (80 vs 100%) and gentamycin (69.1 vs 100%) and also were less sensitive than Mohammed (2005) isolates. This probably, because, Elgadasi (2003) isolates were recoverd from milk treated with three drugs.

Mastitic cow's milk isolates of this study showed resistance to tetracycline (67%). This finding does not agree with Mohamed (2005) who reported that mastitic cow's milk isolates were completely sensitive to tetracycline (100%). The high multi drug resistance observed in mastitic cow's milk isolates of the present study can be attributed to extensive and intensive use of different intramammary preparation for the treatment of mastitis in lactating diary cattle.

Twenty isolates of *E.coli* were obtained from child diarrhea. This result is in agreement with Shears *et al*. (1987), who obtained fifty- one isolates of *E.coli* from stools of thirty- four children in Sudan.

Child diarrhea isolates of the present study showed resistance to amoxicillin (60%) and tetracycline (70%) and most of the strains showed multi- resistance (85%). This result confirms the finding of Shears *et al*., (1987) who stated that sensitivity to six antibiotics, generally available in Sudan, were determined for *E.coli* isolated from children with diarrhea.

Twenty- five (74%) of the children had isolates resistant to at least four of the antibiotics, and seven (20%) had isolates resistant to all six antibiotics. The isolates of child diarrhea in the present study showed complete susceptibility (100%) to nalidixic acid. This finding agrees with Malkawi and Youssef (1998) who stated that all *E.coli* isolates recovered from specimens of children with diarrhea were sensitive to nalidixic acid.

The high prevalence of resistance to tetracycline observed in *E.coli* isolated from animals in this study can be attributed to many factors; the extensive use of oxytetracycline in the treatment of the diseases, Sub- dosing and incomplete duration of treatment.

Multi- drug resistance was shown by most of *E.coli* strains (75.5%) isolated in present study and the multiple drug resistance observed varied from two multiple drug resistant to nine multiple drug resistant. This result is similar to that reported by Mohammed (2005) who observed 12 antibiotics multi drugs resistance among *E.coli* strains isolated from mastitic milk. This finding agrees with Cohen (1992) who reported multi- drug resistance in Escherichia and some others Gram- negative bacteria. Resistant enterobacteriaceae frequently contain multiple plasmids, the larger of which can carry genes for resistance to 10 or more antimicrobial agents (Jacoby, 1991). This may explain the very high multiple- drug resistance of *E.coli* (up to 9 antimicrobial agents) observed in this study. All *E.coli* isolated in this study were resistant to one or more of beta- lactam ring antibiotics. This probably due to the fact that Gram- negative bacteria, including enterobacteriaceae and others produce dozens of different β-lactamases enzymes interfere with the action of beta- lactam antibiotics (Forbes *et al.*, 1998).

The high antibiotic resistance showed by *E.coli* (92.1%) isolated in the present study from human and animal sources may be due to wide misuse of antibiotic in human and veterinary medicine. In addition antibacterial antibiotics may continuously used in foods of animals such as broiler as antimicrobial growth factor.

CONCLUTIONS:

This study showed a high prevalence of antimicrobial drug resistance in *E.coli* isolated from different sources.

Infected poultry isolates showed the highest resistance to tetracycline (86%) and nalidixic acid (85%).

Women urine isolates showed high susceptibility to amikacin (100%), nitrofuranation (100%), gentamicin (75%).

Women urine isolates showed high resistance to tetracycline (75%). Medium susceptibility (50%) to ampicillin, most of the strains showed multi- resistance (87.8%).

Mastitic cow's milk isolates were completely sensitive to amoxicillin (100%), ceftizoxime (100%), chloramphenicol (100%), cephalexin (100%), ciprofloxacine (100%), nitrofuranation (100%), nalidixic acid (100%), gentamicin (100%), norfloxacin (100%), ofloxacin (100%), ampicillin (100%), co- trimoxazole (100%) and pefoxacin (100%), but showed high resistance to tetracycline (67%).

Child diarrhea isolates showed high resistance to amoxicillin (60%) and tetracycline (70%) and most of the strains showed multi- resistance 85%.

All *E.coli* strains isolated in this study were resistant to one or more of beta- lactam antibiotics.

Multi- drug resistance was shown by most of the *E.coli* strains isolated in this study.

RECOMMENDATION

From the results of this work it is recommended that:

1- Further extensive work should be carried out to survey the prevalence of antibacterial drug- resistance to determine the most effective antibiotic.

2- Further studies on antibacterial resistance, multi- resistant and plasmid profile should be conducted on *E.coli* isolates.

3- More research should be carried out to examine genotypic relationships between human and animal *E.coli* isolates.

4- After examination of sensitivity of isolates, antibacterial drugs should be given in full therapeutic doses for adequate period.

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